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PARASITOLOGY

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CONTENTS

No. 1 (JANUARY)

PAGE

DOBELL, CLIFFORD. Researches in the Intestinal Protozoa of Monkeys and Man. IV. An experimental study of the *Histolytica*-like species of *Entamoeba* living naturally in Macaques . 1

WIGGLESWORTH, V. B. Digestion in *Chrysops silacea* Aust. (Diptera, Tabanidae) 73

ROY, D. N. and MAYNE, BRUCE. A Note on the Presence of Supernumerary Lobes in Salivary Glands of Anopheline Mosquitoes. (With Plate I) 77

JOBLING, B. A New Species of the Genus *Raymondia* Frauenfeld (Diptera Pupipara, Streblidae) with a note on *Raymondia quadriceps* Jobling and *R. bedfordi* Ferris. (With 2 Text-figures) . 79

MOGHE, M. A. A Supplementary Description of *Lytocestus indicus* Moghe (Syn. *Caryophyllaeus indicus* Moghe 1925. Cestoda). (With 6 Text-figures) 84

BROWN, F. J. Some Fresh-water Larval Trematodes from Cheshire. (With Plates II and III including 15 figures) 88

BHALERAO, G. D. Two new Trematodes from Reptiles: *Paryphostomum indicum* n.sp. and *Stunkardia dilymphosa* n.g., n.sp. (With 5 Text-figures) 99

LEIGH-SHARPE, W. HAROLD. *Pennella germonia* n.sp. A Parasitic Copepod of *Germo alatunga*. (With 1 Text-figure) . . . 109

FERRIS, G. F. The Louse of Elephants. *Haematomyzus elephantis* Piaget (Mallophaga: Haematomyzidae). (With Plates IV and V and 5 Text-figures) 112

No. 2 (APRIL)

BISHOP, ANN. The Morphology and Method of Division of *Trichomonas*. (With Plates VI and VII including 60 Figures) . . 129

MEHRA, H. R. A New Genus (*Spinometra*) of the Family Lepodermatidae Odhner (Trematoda) from a Tortoise, with a Systematic Discussion and Classification of the Family. (With 6 Text-figures) 157

PAGE

MEHRA, H. R. On Two New Species of the Genus *Astiotrema* Looss belonging to the Family Lepodermatidae Odhner. (With 4 Text-figures) 179

MEHRA, H. R. On a New Trematode *Microderma elinguis* n.g., n.s. (With 1 Text-figure) 191

CANAVAN, WILLIAM P. N. Nematode Parasites of Vertebrates in the Philadelphia Zoological Garden and Vicinity. (With Plates VIII and IX including 25 Figures) 196

BEDFORD, G. A. H. *Nuttalliella namaqua*, a New Genus and Species of Tick. (With Plate X and 1 Text-figure) 230

LEIGH-SHARPE, W. HAROLD. The Appendages of *Basanistes huchonis* (Schrank) (Copepoda). (With 4 Text-figures) 233

BEDFORD, G. A. H. Descriptions of Three New Species of *Tetrophthalmus* (Mallophaga) found on Pelicans. (With 6 Text-figures) 236

SIKES, ENID K. Notes on Breeding Fleas, with Reference to Humidity and Feeding. (With a Chart) 243

MEGGITT, F. J. On Cestodes Collected in Burma. Part II. (With 6 Text-figures) 250

FALCOZ, L. Matériaux pour la Connaissance des Diptères Pupipares. I. (Avec 5 figures dans le texte) 264

MATHESON, ROBERT. Note on the Tick *Ornithodorus talaje* (Guér.-Mén.) 270

No. 3 (JULY)

ARCHIBALD, R. G. and MARSHALL, A. A Study of three Non-furcocercous Cercariae obtained from *Bullinus contortus* in the Sudan. (With 6 Text-figures) 271

RILEY, WILLIAM A. Sarcosporidiosis in Ducks. (With Plate XI) . 282

BISHOP, ANN. A Description of *Embadomonas* n.spp. from *Blatta orientalis*, *Rana temporaria*, *Bufo vulgaris*, *Salamandra maculosa*; with a Note upon the "Cyst" of *Trichomonas batrachorum*. (With Plate XII) 286

TAYLOR, E. LEONARD and PURCHASE, HARVEY S. Do Penetrating Nematode Larvae assist Bacterial Invasion from the Bowel? . 301

PAGE

HARPER, W. F. On the Structure and Life Histories of British Freshwater Furcocercariae. (With 3 Figures in the Text) . . 310

DUKE, H. LYNDHURST. *Trypanosoma gambiense* in Monkeys and Ruminants; Prolonged Infection, Immunity and Superinfection 325

WALLACE, JAMES MONTAGUE and WORMALL, ARTHUR. Red-cell Adhesion in Trypanosomiasis of Man and other Animals . . 346

ACKERT, JAMES E. The Morphology and Life History of the Fowl Nematode *Ascaridia lineata* (Schneider). (With Plates XIII, XIV, and 25 Text-figures) 360

KEARNS, H. G. H. The Larval and Pupal Anatomy of *Stenomalus micans* Ol. (Pteromalidae), a Chalcid Endoparasite of the Gout-fly of Barley (*Chlorops taeniopus* Meig.), with some Details of the Life History of the Summer Generation. (With 5 Figures in the Text) 380

MANTER, H. W. Some Digenetic Trematodes of Marine Fishes of Beaufort, North Carolina. (With 25 Figures in the Text) . 396

TIEGS, O. W. Note on the Occurrence of Sarcocystis in Muscle of Python. (With 4 Figures in the Text) 412

No. 4 (OCTOBER)

HEYDON, G. M. Some Common Queensland Mosquitoes as Intermediate Hosts of *Wuchereria bancrofti* (*Filaria bancrofti*) . . 415

REES, GWENDOLEN. Some Observations and Experiments on the Biology of Larval Trematodes. (With Plate XV and 5 Text-figures) 428

WIGGLESWORTH, V. B. Haematin in the Tracheae of Blood-sucking Insects: an Artefact 441

MUKERJI, S. The Process of Copulation in *Phlebotomus argentipes* Ann. & Brun. (With 1 Figure in the Text) 443

ANDRÉ, E. Sur la Piqûre du *Nabis apterus* 445

LUBIMOV, M. P. *Gongylonema macrogubernaculum* n.sp. (Nematode) from Monkeys. (With 3 Figures in the Text) 446

HOARE, CECIL A. Studies on *Trypanosoma grayi*. III. Life-Cycle in the Tsetse-fly and in the Crocodile. (With Plates XVI–XIX and 3 Text-figures) 449

PAGE

SWAN, D. C. On a New Species of *Ixodes* (*Ix. hydromyidis*) from Western Australia. (With 1 Figure in the Text) . . . 485

HINMAN, E. HAROLD. *Pediculus* (*Parapediculus*) *atelophilus* Ewing 1926 from the Red Spider Monkey, *Ateles geoffroyi*. (With 2 Figures in the Text) 488

MURRAY, FLORENCE V. Gill Trematodes from some Australian Fishes. (With Plates XX–XXII and 1 Text-figure) . . 492

DAUBNEY, R. and HUDSON, J. R. Nairobi Sheep Disease. (With 6 Charts) 507

COATNEY, G. ROBERT. On the Biology of the Pigeon Fly, *Pseudolynchia maura* Bigot (Diptera, Hippoboscidae) . . . 525

BAYLIS, H. A. On the Structure and Relationships of the Nematode *Capillaria* [*Hepaticola*] *hepatica* (Bancroft) 533

Index of Authors 545

Index of Subjects 547

VOLUME XXIII JANUARY, 1931 No. 1

RESEARCHES ON THE INTESTINAL PROTOZOA OF MONKEYS AND MAN.

IV. AN EXPERIMENTAL STUDY OF THE *HISTOLYTICA*-LIKE SPECIES OF *ENTAMOEBA* LIVING NATURALLY IN MACAQUES.

BY CLIFFORD DOBELL, F.R.S.

National Institute for Medical Research, London, N.W. 3.

Every complete devotion to an idea yields some profit, even though it be different from that which was expected.
F. NANSEN (1911), *In Northern Mists*, vol. I, p. 3.

CONTENTS.

PAGE

I. Introduction 2
II. Methods and Materials 5
Methods of cultivation 5
Data relating to monkeys 7
Notes on kittens 10
Technique of inoculation 12
III. Experiments and Results 14
1. Experimental Infection of Kittens with *E. histolytica macacorum* . . . 15
(i) Direct attempts with amoebae and cysts in faeces 15
(ii) Indirect attempts with cultures 16
Strain *B.* 17
(iii) Further experiments with Strain *B* 19
Case of Kitten 15 21
Case of Kitten 16 24
(iv) Further attempts with cysts in faeces and amoebae in cultures . . 25
Case of Kitten 28 26
Attempts to transmit Strain *K.*28c. 26
Case of Kitten 31 28
Strains *BX.* and *BC.* 30
Summary 30
2. Experimental Infection of Macaques with *E. histolytica macacorum* . . . 31
(i) Transmission from *sinicus* to *sinicus* (Strain *K.*28c.) 32
(ii) Transmission from *rhesus* to *sinicus* (Strains *Jc.*, *JSc.*) 34
(iii) Transmission from *nemestrinus* to *rhesus*, and thence to *sinicus* (Strains *Nc.*, *NRK.*, *NRS.*) 36
Summary 40
3. Experimental Infection of Macaques with *E. histolytica hominis* . . . 41
Strain *D.* 41
(i) Transmission of Strain *D.* to *M. sinicus* 41
Strain *DM.* 42
(ii) Transmission of Strain *D.* from *sinicus* to *rhesus* 42
Strain *DMR.* 44
(iii) Transmission of Strain *D.* to *sinicus* and *rhesus* after prolonged cultivation (5 years) 44
Strains *DS.* and *DRK.* 47
Summary 49

III. Experiments and Results (*cont.*) PAGE
4. Supplementary Experiments 49
(i) Infectivity of various strains (*D.*, *F.*, *P.*, *DM.*, *DR.*, *DRK.*, *DS.*, *DS.*[2]) for kittens 49
(ii) Ingestion of red blood-corpuscles by *E. histolytica* 55
Summary 60
IV. Discussion 60
General Conclusions 70
References 71

I. INTRODUCTION.

ELEVEN years ago, in the course of work on the amoebae living in Man, I found it necessary to consider the intestinal amoebae of Monkeys[1]. After briefly reviewing the literature dealing with these organisms, and after emphasizing *inter alia* the apparent resemblances between certain amoebae described from various simian hosts and the pathogenic amoeba (*Entamoeba histolytica*) inhabiting men, I concluded tentatively[2] that it was "by no means impossible" that all these species of *Entamoeba* "are really identical"; but I was careful to add that "At present [1919] there are not sufficient data to determine this point, and the question can only be decided by further observation and experiment." The following paper is the outcome of an attempt—which has engaged the major part of my time for the last six years—to make such observations and experiments, as accurately and decisively as possible, within a small and definite area of this limitless field of research[3].

To a casual onlooker it may well seem absurd to devote years of intense effort to the solution of a problem which apparently possesses merely systematic and academic interest. Why should one worry about the *histolytica*-like species of *Entamoeba* which occur in so many different monkeys? Why not leave them in their present confusion, and devote oneself to more profitable studies? Why not go on calling them by the various fancy names now in vogue?—for of what real use or interest can it be to determine whether these amoebae belong to one species or to one thousand?

But every serious student must, I think, agree with me that this inquiry is neither unnecessary nor unprofitable. The proper answers to questions such as those just asked have already been indicated in the General Introduction to this series of researches[4], but they have so direct a bearing upon the work now about to be recorded that I venture to repeat them in slightly different words.

Since *E. histolytica* is—or may be—a cause of painful disease in Man, it is obviously to his own interest to learn everything possible about this organism. Its natural history must be known in every detail before Man can hope to rid himself from its ravages and obtain complete control over its activities—in so far as they concern himself. Consequently, we want to know—and know

[1] See Dobell (1919), pp. 131–133. [2] *Ibid.*, p. 133.

[3] Preliminary notices of the present work have already been published in the annual *Reports of the Medical Research Council* for 1924–25, 1925–26, 1926–27, 1927–28, and 1928–29.

[4] See Dobell (1928).

certainly—whether the several *histolytica*-like species of *Entamoeba* frequently found in other animals (monkeys, rats, pigs, and so forth) are, or are not, of the same species as our own parasite. We want to know this not only for the sake of advancing zoological knowledge, but also for our own protection: we want accurate information regarding the host-distribution of *E. histolytica* in nature, because such knowledge is not merely of academic interest but also of practical personal importance.

We cannot learn much more about *E. histolytica* by pursuing the old lines of simple observation and morphological comparison. We must now make experiments; and our experiments must, of necessity, often be made upon living animals. For the study of a human parasite, the ideal experimental host is obviously Man himself; but since we cannot do dangerous researches on our fellow-beings, we must perforce use other animals instead. Consequently, the discovery of some animal which is naturally infected with *E. histolytica* would, in itself, be of inestimable assistance to all future investigation. I hoped to find such a natural host of the parasite among the Primates, and I confess I have not been disappointed[1].

Although I planned my work in such a way that it might also furnish data for solving many minor problems, the main object of the part here described was quite simple. I wished to ascertain with certainty whether the *histolytica*-like species of *Entamoeba* which occurs naturally in monkeys of the genus *Macacus* (= *Macaca* = *Silenus*) is, or is not, specifically identical with *E. histolytica* of Man.

I shall endeavour to present my evidence relating to this inquiry as objectively and concisely as possible, yet with sufficient detail to enable the reader to draw his own inferences independently. In perusing the works of most previous authors I have generally found their accounts unconvincing; because they have failed to record—and, apparently, in many cases even to ascertain—particulars which are of vital importance for the interpretation of their observations and experiments[2]. Consequently, if I now present my own results with a degree of detail which may seem at first sight over-elaborate, it is none the less intentional. My aim is to record every relevant datum which may be of use to other students of the same subject.

I have already committed myself to the opinion[3] that *E. histolytica* (of Man) and the closely comparable organisms found in Macaques are specifically identical, but the present memoir provides the chief premisses for this conclusion. I have therefore entitled this instalment a study of "the *histolytica*-like species of *Entamoeba* living naturally in Macaques"—thereby enunciating

[1] Some of my grounds for believing that a tame macaque can serve as substitute for a man—in the study of human intestinal protozoa—will be found in the following pages. Other evidence will be presented later.

[2] For example, several writers claim to have infected "a monkey" (no species or genus recorded) with *E. histolytica* experimentally—but without mentioning any measures taken to insure that the animal was uninfected before inoculation.

[3] Dobell (1927, 1928), Dobell with Bishop (1929), *et alibi*.

the problem which it attempts to solve: but I have been compelled, for want of any less objectionable name, to call this parasite "*E. histolytica*" in the body of the work—thus forestalling the conclusion ultimately arrived at. I am now convinced that all the *histolytica*-like *Entamoebae* which I have studied in various Macaques are specifically indistinguishable from one another and from the true *E. histolytica* of Man, though I am equally certain that there are many different strains or races of this species inhabiting all these hosts. To avoid confusion, therefore, I have arbitrarily designated all strains derived from Man as *E. histolytica hominis*, and all strains from Macaques as *E. histolytica macacorum*[1]—names which are not now proposed for any new and valid varieties or subspecies under the International Code of Nomenclature, but which are employed merely for convenience. By adopting these self-explanatory descriptive designations, it has been possible to preserve a distinction between similar amoebae of human and simian origin, while at the same time recognizing their probable specific identity.

It is obvious that the systematic affinities of *E. histolytica hominis* and *E. histolytica macacorum* can be exactly determined by only one method—the method universally employed by all sound zoologists since the time of Aristotle. All the amoebae in question must be studied in detail, both morphological and physiological; and their characters and properties, as so revealed, must then be compared or contrasted in order to prove their identity or their diversity. I have already published[2] the chief results of my morphological studies in the present connexion (which led me to the conclusion that, as regards structure and life-history, all these organisms are indistinguishable); and I shall here record my observations on some of their principal physiological properties, as ascertained by simple experiments. These lead me to the same conclusion again—a conclusion with which I trust that every attentive and reasonable reader will concur.

Unfortunately, the experiments recorded in the following pages are not very numerous. They have been sufficient to convince me of the truth of my general conclusions, though from a statistical standpoint they are manifestly inadequate. In extenuation of this deficiency, however, I would beg the reader to bear in mind that all such experiments are extremely laborious, and require many months—and sometimes years—for their proper performance. To keep monkeys—and even kittens—alive and healthy in strict confinement, for as long as needful, is in itself no light task: while to isolate, study in detail, and maintain *in vitro* even half-a-dozen strains of *E. histolytica* for only a few years, is also almost a whole-time occupation for one person. Moreover, whilst this work has been in progress I have had to conduct many similar experiments

[1] As I am unable to distinguish between the forms of "*E. histolytica*" living in the three different species of *Macacus* which I have studied (*rhesus, sinicus, nemestrinus*), I include them all under this common name. It seems to me unnecessary to subdivide "*E. h. macacorum*" into "*E. h. macaci-rhesi*," "*E. h. macaci-sinici*," etc., since no solid grounds—either morphological or physiological—exist at present for drawing such distinctions.

[2] See Dobell (1928).

simultaneously—each of which has demanded the same attention—and I have had perforce to carry out the whole single-handed, with inadequate accommodation for keeping and isolating my animals, and often in the face of almost insuperable material difficulties. Only those who spend an equal time in personally performing every part of an equal number of experiments with equal care, over a like period and with the same limited facilities, can fully appreciate the hardships and hazards of such a piece of work. I am conscious that my researches are incomplete, but I hope to repair some of their more obvious shortcomings at an early date. Meantime I can but crave the reader's indulgence for inevitable defects which are even more evident to myself than they can be to him.

II. METHODS AND MATERIALS.

These researches have been performed with various strains of *E. histolytica* [*sensu lato*] derived from ten macaques and two human beings. All strains used were morphologically typical, with cysts measuring 11–14μ in diameter. Most of them have been cultivated for divers periods in "pure" culture (*i.e.* as pure cultures of the amoebae in question—free from all other protozoa, but accompanied by an unknown though stable mixture of bacteria), and have been introduced either directly (from the faeces of their host) or more usually indirectly (by way of cultures) into monkeys and kittens: and when infection has resulted, they have then been recovered in "pure" culture once more, and cultivated and studied further. The actual experiments will be described in the next section; but I shall here give first a few particulars regarding the methods of cultivation employed, the experimental animals used (monkeys and kittens), and the technique adopted for their inoculation.

Methods of Cultivation.—The methods which I have used throughout are various modifications of the technique discovered by Boeck and Drbohlav in 1924[1]. They were devised by Dr P. P. Laidlaw and myself in 1924–25; but as they have already been published[2], I need say no more about them here. I am aware, of course, that many "improvements" have since been proposed; but as they offer no obvious advantages, and appear to be mostly based upon misconceptions or ignorance of our work, I have not adopted any of them. By our methods, which are all well tried and with which I am thoroughly conversant, I can cultivate any strain of *E. histolytica* from any desired host for as long as I please, and can obtain any required stage in its life-history *in vitro* at any moment. Consequently, I have had no need to use other methods which seem to me less certain and which have been less extensively tested by other workers.

It is now comparatively easy for anybody to cultivate *E. histolytica*, by any of the well-known methods, for any length of time in the active (amoeboid) form: but it is not always so easy for him to obtain ripe normal cysts, in any quantity required, from any strain at any given moment. I have already

[1] See Boeck and Drbohlav (1925).

[2] See Dobell and Laidlaw (1926*a*); Dobell (1927); Laidlaw, Dobell, and Bishop (1928); Dobell (1928).

described how this can be done in the case of one particular strain (*K*.28 c.) by adding rice-starch to the cultures at an appropriate stage[1]; and a similar method has been found applicable to *all* strains—of whatever origin—which I have yet studied.

My work hitherto has shown conclusively, however, that for encystation to be able to occur in a culture of *E. histolytica*, certain bacteria must be present. If these bacteria are absent, the amoebae never encyst—no matter how much starch they may eat, or how much the other cultural factors may be varied. In the case of Strain *K*.28 c. the necessary bacteria are present among the natural concomitant flora, so that on the simple addition of rice-starch to any culture, encystation can be readily induced. Moreover, any other strain which, when isolated with its own individual bacterial flora, does not behave like Strain *K*.28 c., can be made to do so by adding the bacteria accompanying this strain. I made this discovery soon after I isolated Strain *K*.28 c. in 1926, and it has been of the greatest service in all my subsequent work: for it has enabled me to obtain any quantity of cysts at will, at any desired moment, from every strain of *E. histolytica* which I have yet cultivated. Even strains which have been kept in starch-containing media for several years in the amoeboid state—without undergoing encystation at any time—can be induced to encyst promptly and copiously by adding the necessary bacteria to the cultures[2].

I have now isolated and studied a large number of strains of *E. histolytica*, and maintained many of them *in vitro* for considerable periods. When once a strain has been isolated, and has reached a state of stable equilibrium with its concomitant bacterial flora, its cultural characters remain constant. Its behaviour can therefore be predicted, and it can be propagated under given conditions *ad libitum*. Successful maintenance *in vitro*, however, demands constant attention to details; and special care must be exercised in the preparation and sterilization of the culture-medium and all its ingredients at every stage, because accidental contamination with foreign bacteria may entirely change the properties of a strain and even lead to its extinction. All this has been said before[3]; but I repeat it because it is of vital importance for every worker who would undertake experiments like those about to be described—experiments

[1] See Dobell (1928), pp. 370 *et seq.*

[2] The bacteriological analysis of the complex flora naturally accompanying any cultivated strain of *E. histolytica* is extremely difficult and laborious, and I am therefore not yet able to give a complete account of all the bacteria present with Strain *K*.28 c., or to state definitely which or how many of them are the "necessary" species just alluded to. I can only say at present that Strain *K*.28 c. contains *all* the necessary bacteria, and that if all the bacteria accompanying this strain be added to any other strain, it will then behave culturally exactly like *K*.28 c.—growing in the same way, and undergoing encystation and excystation under precisely similar conditions. I may add, however, that the bacteria necessary for encystation appear to have a specific relation to *E. histolytica*: for I have found that they do not influence cyst-formation in any way when similarly introduced into cultures of *E. coli*, *E. gingivalis*, *Endolimax*, *Dientamoeba*, *Balantidium*, or *Chilomastix*.

[3] Cf. Dobell and Laidlaw (1926*a*), Dobell (1927, 1928), etc.

which involve continuous cultivation of numerous pure strains of *E. histolytica* for months and sometimes for years.

As the continued cultivation of many strains for considerable periods takes up much time, I have latterly adopted a simple expedient which may also be serviceable to other workers. I now cultivate various strains—which I desire to keep for further study—discontinuously. I cultivate the active amoebae (in the usual ways) for a short period (usually about a week), and then make them encyst (by the method just described). I then store the cysts in the ice-chest, at a temperature of about 10° C. (varying from *ca.* 8° to 12°) for a month —a time which I have found by trial to be absolutely safe. After a month (28–31 days) in the ice-chest, the cysts are hatched and the amoebae cultivated for another week or so. They are then made to encyst again, and the process is repeated as long as necessary. In this way I have been able to maintain many strains simultaneously for any length of time with complete safety, and with a minimum of labour and great economy of culture-medium. Strains so propagated differ, in my experience, in none of their properties from parallel strains kept under continuous cultivation at 37° C.

To avoid any misunderstanding, I must note here that whenever—in the following pages—I say that I "isolated" a strain of *E. histolytica* on a given date, I mean that I obtained the necessary material and initiated cultures (from which the strain was ultimately derived) at that time. The actual isolation—separation from concomitant protozoa and *Blastocystis*—frequently occupied several subsequent weeks. All my monkeys harboured *Blastocystis* and more than one species of intestinal protozoon, and the purification of every strain of *E. histolytica* cultivated from them—though not here described in detail—has been one of the most onerous essentials of the present investigation.

Data relating to Monkeys.—All the monkeys used for this work were Macaques, obtained originally from dealers in London and discarded by fellow-workers in the Institute[1]. Some of these animals—the most important —were members of the small family which I have maintained since 1924: the rest were odd specimens acquired by chance and studied only incidentally. A few particulars regarding my own monkeys have already been recorded[2], but I must now give some additional information about each individual employed in the present researches.

Two macaques, which served merely as sources of material, were placed at my disposal after death; and two others were available for only short periods during life. But the remainder—those forming my own monkey-family—were the sole animals used for experimental purposes. These were all healthy, vigorous, thoroughly acclimatized, and perfectly tame and manageable in my hands. I made no experiment upon any monkey until it had been in my care and under my daily personal observation for at least a year

[1] See Dobell (1928), pp. 359 *et seq.*

[2] See Dobell (1928, I), and Dobell with Bishop (1929).

and a half previously; and the intestinal protozoa of every individual were investigated exhaustively before it was subjected to any of the experiments here recorded.

A complete list of the macaques studied is given in Table I, together with a few relevant particulars—including the total natural amoebic infections (ascertained by prolonged microscopic and cultural investigation) of each individual. All these animals were also infected naturally with various other intestinal protozoa; but as these concomitant organisms—though studied in detail in every case—do not now concern us, they need not be considered here.

Table I.

Name	Species	Sex	Intestinal Amoebae
(1) **Polo**	*M. sinicus*	♂	*Entamoeba histolytica*; Endolimax nana*
(2) **Jacko**	*M. rhesus*	♂	*E. histolytica; E. nana*
(3) **Mungo**	*M. sinicus*	♂	*E. histolytica; E. nana*
(4) **Susanna**	*M. sinicus*	♀	*E. histolytica, E. coli; E. nana*
(5) **Rosa**	*M. rhesus*	♀	*E. histolytica, E. coli; E. nana*
(6) **Bonar**	*M. sinicus*	♂	*E. histolytica; E. nana*
(7) **Samson**	*M. rhesus*	♂	*E. histolytica; E. nana*
(8) **Delilah**	*M. rhesus*	♀	*E. histolytica; E. nana*
(9) —	*M. rhesus*	♀	*E. histolytica; E. nana*
(10) —	*M. nemestrinus*	♀	*E. histolytica, E. coli; E. nana*

* Including, throughout the table, both strains with large cysts (11–14μ) and those with small (7–9μ).

It will be evident from Table I that the monkeys studied were of three different species—*Macacus sinicus* (4 individuals), *M. rhesus* (5), and *M. nemestrinus* (1). I use the old-fashioned and familiar names adopted by Forbes (1897) for these species, in preference to more recent and less-known designations[1]; though I am aware that reasons can be given for changing the genus *Macacus* to *Macaca*, and also for supplanting it more or less completely by *Silenus*. To avoid all possible confusion, therefore, I would expressly note that the species of macaque which I call "*M. sinicus*" is the Southern Indian Bonnet-monkey (now called *Macacus radiatus*, *Macaca radiata*, *Silenus radiatus*, or *Silenus sinicus* by different writers), and not the Ceylonese Toque-macaque (called *Macacus pileatus* by Forbes) to which the specific name *sinicus* has recently been transferred. By "*M. rhesus*" I mean the common Bengal Macaque—*forma typica*, not a related species or subspecies—and by "*M. nemestrinus*" the Malayan Pig-tail. I have, to the best of my ability, accurately identified every monkey whose intestinal protozoa I have studied.

The following further data are recorded because they may be necessary for the interpretation of my findings:

[1] I note this again—for I mentioned it in an earlier instalment (Dobell, 1928, p. 359)—in order to prevent misunderstanding. Unfortunately at least one writer (Hirst, 1929) has already found some difficulty in interpreting my nomenclature.

My original family consisted of four monkeys—Polo, Mungo, Susanna, and Jacko (3 *M. sinicus* and 1 *M. rhesus*), all very young animals. They came to the Institute, shortly after their arrival in England, at the end of May 1924 and came into my possession[1] in August of the same year. Jacko was an animal kept as an untreated control to the experiments performed[2] upon the other three, and was therefore "normal" when I acquired him. From August 1924 onwards I studied all the intestinal protozoa of these four monkeys in great detail. Jacko was ultimately sacrificed on 15 February 1928, Polo on 28 July 1928, and Mungo on 8 August 1928. Susanna is still alive and under observation and experiment.

Samson and Delilah (both *M. rhesus*) belonged to Dr W. Mair[3], who kindly permitted me to investigate their infections. The former I was able to examine only casually during life (in August 1924) though I obtained his intestines for *post-mortem* study when he was killed (29 October 1924). But Delilah—a large and savage female—was in my keeping during part of the winter of 1924–1925, and I was therefore able to investigate her intestinal protozoa pretty thoroughly[4]. She was killed on 27 January 1925 (by Dr Mair), and afforded me valuable material for cultivation and *post-mortem* examination. These two monkeys came to the Institute originally in November 1922, and had thus been in captivity for about a couple of years before I examined them. They were well-grown adults, caged separately, who had occasionally been in contact with one another but not with other monkeys.

Bonar (*M. sinicus*)—to me a very interesting and important monkey—also belonged originally to Dr Mair. He came to the Institute[5] in November 1923, and I first examined him in August 1924. He was added to my family in January 1925, and was under my constant supervision until I killed him on 8 September 1927.

Rosa (*M. rhesus*) was originally a very young female who came into my possession in June 1927. She arrived at the Institute in October 1926, and had been used for experiments (on respiration) by Dr J. Argyll Campbell before I acquired her. She is still alive and in excellent health—notwithstanding the numerous experiments to which she has been subjected.

The unnamed female *M. rhesus* (No. 9 in Table I) was an animal which died (on 5 May 1925) shortly after entering the Institute and before any experiments could be made with her. Her owner, Dr W. J. Purdy, kindly

[1] For these animals I am indebted to Dr P. P. Laidlaw, F.R.S.

[2] By Dr Laidlaw. The experiments gave negative results, and so far as I can judge had no harmful effects of any sort.

[3] I regret that in an earlier instalment (Dobell, 1928, pp. 360, 367) Dr Mair's initial is—by an unfortunate oversight—erroneously given as "J." instead of W.

[4] Between 13. viii. 24 and 27. i. 25 I examined Delilah's faeces microscopically on 13 occasions, and I isolated and studied several strains of *E. histolytica* and other protozoa from her both before her death and *post mortem*.

[5] Whilst he was in Dr Mair's possession Bonar was kept in the same room with Samson and Delilah, but in a separate cage.

allowed me to have her intestines for *post-mortem* study. Unfortunately the tissues were too decomposed for histological investigation, but in the intestinal contents the protozoa were still alive and active. I isolated and studied—*inter alia*—a pure strain (*T.*) of *E. histolytica* from this macaque.

Only one specimen of *M. nemestrinus* has hitherto been available (No. 10)—a full-grown female used for surgical experiments by Sir Charles Ballance and killed by him under chloroform on 3 August 1927. I obtained the body immediately after death, and was thus able to make a detailed *post-mortem* examination of the intestines. This animal had been in the Institute (caged alone) since May 1926. I studied and cultivated all her intestinal protozoa, and isolated from her a pure strain (*Nc.*) of *E. histolytica* which figures in the following experiments.

All my experimental macaques were tamed, trained, and tended by myself. They were under my daily personal observation throughout, and no part of any experiment was ever entrusted to assistants. I personally collected and examined every specimen of faeces whose protozoal content is here recorded, and can therefore guarantee its authenticity. I kept all my monkeys as pets—not as wild animals—and during the period of the present experiments I have devoted far more attention to their diet, health, happiness, and general well-being, than I have to my own. I am therefore confident that all the monkeys used in these researches were as "normal" as possible, and that my experiments were not vitiated by fallacies due to the use of abnormal or unhealthy subjects.

All my own macaques have been kept in separate cages, and allowed to associate only when I considered it safe for them to come in contact. Owing to lack of adequate accommodation, however, I have been compelled to house them all in the same room (the only place where I could keep them separate from all other animals in the Institute). But throughout every experiment each monkey has otherwise been completely isolated from its fellows, and every precaution has been taken to prevent accidental infections. This has only been possible by exercising the strictest personal supervision and unremitting vigilance at all times.

Notes on Kittens.—All the kittens used in this work were also kept as pets[1], and cared for by myself. Every animal was under my daily personal observation during the whole of every experiment. With the exception of a few individuals used in the earliest experiments, moreover, they were all born—from known healthy mothers—either at the Farm belonging to the Institute or in the Institute itself, and were under my own care from earliest infancy.

In my experience—which now covers some 200 individuals—almost all London kittens are naturally infected with coccidia, ascarids, cestodes, and

[1] My kittens—like my monkeys—had individual names by which they were known to me: but as I shall here have to record experiments made upon about 40, I think it best to refer to them merely by their numbers.

other more or less pathogenic intestinal parasites[1]. Those obtainable from dealers are usually infected heavily, and are so sickly and verminous that they are useless for any accurate experiments with *E. histolytica*. Moreover, since young kittens are more susceptible to infection with this amoeba than older animals, it is desirable—in work like the present—to know (not guess) the ages of one's experimental subjects. Consequently, I took every precaution to insure that all my own kittens were both young and healthy. All those mentioned in this memoir were ascertained to be in really good condition before they were subjected to any experimental treatment; and I knew the exact ages of the majority, because they were born and reared in my animal-room and were under my eye from birth.

As soon as they were old enough, I kept all my kittens separately in specially devised cages which enabled me to study all their dejecta individually. To keep them happy, however, I frequently allowed them to play together.

At one time it appeared to me possible that susceptibility to infection with *E. histolytica* might depend, to some extent, upon the diet of the experimental animal. I therefore made several experiments with kittens fed in different ways; but as the results were entirely negative, I need not record them here. I have latterly fed all my kittens, after weaning, on nothing but boiled fish and pasteurized milk—a diet upon which they thrive exceedingly.

Most of my kittens were 6–10 weeks old when first used for experiment; but I have occasionally used older animals (and with successful results—as in the case of Kitten 15). I must also point out that I have more than once used the same kitten for more than one experiment—if the first had been conclusively negative. I am aware that objections can be urged against this procedure, but to me they are unconvincing. No good evidence has yet been adduced to prove that any kind of immunity can be induced in any animal by unsuccessful inoculation with *E. histolytica*. Those writers who speak of "cross-immunity" or "acquired immunity" in kittens which have been unsuccessfully inoculated previously, or which have spontaneously recovered from infection, appear to forget that cats normally become increasingly difficult to infect as they grow older. To forestall any possible objections on this score, however, I may also point out that in the present work I have drawn no definite conclusion from any experiment performed with any kitten employed in a previous experiment, unless it is supported by similar evidence derived from absolutely untainted animals.

I record the foregoing particulars because they seem important, and also in answer to the criticisms of a recent American author[2]. This writer—who has

[1] They are also frequently subject to mange and ringworm, and generally infested with fleas.

[2] "Studies of the clinical course of amebiasis in kittens and of its pathology have not in any instance been adequately pursued. Controls were never kept, though the kittens were usually purchased in the streets. Such animals are undernourished and usually heavily infected with nematodes, cestodes, coccidia and ectoparasites. Kittens are subject to pneumonia and bacillary

no personal knowledge of my methods—stigmatizes the observations of all other workers because they appear to him to have been made upon unhealthy and insufficiently studied kittens. I therefore state here explicitly that his censure cannot be justly applied to any experiments which I have ever made.

The Technique adopted for the Inoculation of Monkeys and Kittens.—A few words must be added here regarding the methods which I have used in my attempts to transmit *E. histolytica* to kittens and monkeys, because they differ in some ways from those of other workers.

Since it is now possible to obtain "pure" cultures of *E. histolytica*—unmixed with any other protozoa—and to obtain cysts of any strain *in vitro* at will, I have attempted to infect all my macaques by means of culture-cysts administered by the natural route *per os*. Cysts of known origin, belonging to carefully studied strains, have been swallowed in a natural manner by every experimental monkey, and I have always made sure that they were not regurgitated or rejected afterwards[1]. This technique is, in my opinion, by far the best. I have never tried to infect any monkeys by means of faecal material—containing, in addition to *E. histolytica*, unknown admixtures of protozoa and other things—forced into the stomach or up the rectum. I regard all such procedures as now out-of-date.

It has been my practice to test the viability of all material used for inoculation into animals—whether it contain amoebae or cysts—by making cultures from small samples at the same time; and I also usually fix and stain a little of the inoculum for detailed microscopic study. Such safeguards may seem superfluous, but in my opinion they are not. In an early experiment (not here described) I once obtained a "negative" result after feeding a monkey on cysts which appeared to be normal in every way: but the sample inoculated simultaneously into a culture-tube produced no amoebae on incubation, and the stained specimen revealed that the cysts were, in reality, all imperfectly developed or abnormal. On repeating the experiment with other cysts—which hatched normally *in vitro*, and were found cytologically to be perfect—the same monkey was readily infected with the same strain of amoebae. This experience was, to me, so instructive, that it seems worth recording; and it confirmed me in the belief that, in work of this sort, no possible precaution should ever be neglected.

One of the greatest obstacles facing every worker who would attempt to infect macaques with *E. histolytica* of human origin is the difficulty of obtaining "clean" (*i.e.* uninfected) monkeys for experimentation: and almost

dysenteries, particularly during the period when most susceptible to amebic dysentery. And yet it appears from the protocols that careful examination of the contents of the stomach and the small intestines were [*sic*] not made. The aforementioned parasites were not noted as being present. Small kittens adapt themselves poorly to solitary confinement in wire cages; frequently the controls die, sometimes before the experiments are under way" (Rees, 1929, pp. 3–4). From his own account it appears that Rees's kittens were "purchased on the streets" (p. 5), and afflicted with all the disabilities which he charges against every other worker.

[1] Cf. Dobell with Bishop (1929), p. 450.

all previous work in this direction has been vitiated by failure to solve—or even to realize—this fundamental problem. My own solution of it was effected by the laborious method of curing my animals of their natural infections with *E. histolytica macacorum*—before experiment—by treatment with emetine. In my experience this method is certain and safe, though extremely tedious; but as my work in this connexion has already been described[1] I need say no more about it here. All the monkeys used in the present researches were made "clean" by previous emetine-treatments which are recorded in detail either in the following pages or elsewhere[2].

The methods which I have used can be practised, of course, with properly trained macaques only. All my experimental monkeys were previously taught to swallow anything which I chose to give them from a pipette. Their education required almost infinite patience; but the time spent upon it was not wasted, because it greatly facilitated all my subsequent experiments. I never had the slightest difficulty in making any experimental monkey ingest as many cysts as I desired at any required moment.

In my earlier experiments with kittens, I injected faecal material (from monkeys) *per anum*: but all such material was very carefully controlled beforehand, and its protozoal content exactly determined. Afterwards, when I had mastered the methods of cultivating *E. histolytica*, I used culture-material for inoculation exclusively (except in a few special experiments designed to test the infectivity of natural cysts from faeces).

It is the easiest thing in the world to get a kitten which is happy and unafraid to swallow anything put into its mouth: and consequently anybody who knows how to handle these animals can have no difficulty in administering cysts *per os*. My own method is to place the kitten, which I wish to inoculate, on its back in my lap: I then fondle it for a few moments; and while it is purring and amused I gently insert a pipette containing the infective material into its mouth, and discharge the contents as far back as possible. As soon as the kitten feels something present in its pharynx, it swallows spontaneously. The whole operation takes only a few minutes, and the kitten is quite unconscious that anything unusual has happened. To my mind, the administration of cysts through a tube forced into the stomach of a terrified animal is as unnecessary as it is brutal.

In experiments devised to determine whether amoebae introduced into the colon are infective, it is obviously important to use really good material and to give it a fair chance. The old-fashioned method of introducing large quantities of supposedly infectious faecal matter—containing comparatively few amoebae, many of which are already dead or degenerate—is no longer

[1] See Dobell with Bishop (1929).

[2] Details regarding dosage, dates, etc., are given for the express purpose of supplying fellow-workers with particulars whose lack, in the publications of others, has inconvenienced me in my own researches. To interpret the findings in such experiments properly, one needs exact data—not inferences drawn by individuals from their own undisclosed experience.

necessary: nor is it now necessary to stitch up the kitten's anus or plug it with cotton and collodion, or to ligature its rectum after laparotomy in order to prevent immediate evacuation of the inoculum. I have therefore used throughout a method which seems to me scientific, and which is also extremely simple. I inject a small quantity (about 2 c.c.) of a rich culture of the required amoebae into the caecal end of the colon of the experimental kitten by means of a glass tube—of appropriate length, and attached to a "Record" syringe—inserted into the anus: and I carry out the whole operation in the hot-room at the Institute (where a temperature of 37° C. is maintained constantly) in order that the amoebae may not be injured by cooling. This small but highly concentrated inoculum, discharged at the appropriate spot, is always well retained and never fails to infect—in my experience—provided that the strain of amoebae used is infective, and the inoculated kitten susceptible.

Recent workers in America have advocated a method of inoculation which involves ligation of the colon at both ends—after laparotomy—and injection of the infecting material through its wall. The unfortunate kitten is then starved for two or three days, when it dies or is killed. It is claimed that infection can be more readily induced by this procedure than by other methods, but to my mind it is not only revoltingly cruel but also well calculated to defeat its object. In my own experiments I have simply tried to discover whether the strains of *E. histolytica* studied are capable of parasitizing healthy kittens, when introduced into their normal large intestines; and I have purposely made no attempts to ascertain whether ulceration and temporary infection can be produced by tying off parts of the bowels of moribund animals, and injecting amoebae into the isolated portions. My technique itself causes—designedly—neither pain nor injury to the kitten.

III. EXPERIMENTS AND RESULTS.

I have made many attempts—both successful and unsuccessful—to transmit various strains of *E. histolytica* to kittens and monkeys. These experiments have been performed as opportunity occurred during the last half-dozen years—some of them running concurrently, others being widely separated in time. It has never been possible to conduct any series of related experiments, as a self-contained and continuous whole, to its final conclusion at any one period; for many experiments have necessarily had to be carried out simultaneously with others forming parts of other series. Consequently, many of the "series" of experiments, as here presented, overlap one another—later members of a "first" series having sometimes been performed long after a "second" or "third" was completed. I note this so that the reader may not be misled by apparent discrepancies in the dates and other details recorded. The order in which the results are now given is a more or less logical order, adopted for the purpose of exposition, and has no regular relation to the time of the actual experiments.

1. EXPERIMENTAL INFECTION OF KITTENS WITH *E. HISTOLYTICA MACACORUM*.

My attempts to infect kittens experimentally with strains of *E. histolytica* derived from Macaques fall into four series (i, ii, iii, iv), which will now be described. Supplementary experiments, designed to elucidate various special points which arose in the course of these and subsequent investigations, will be relegated—for convenience—to a later section[1].

(i) *Direct Attempts with Amoebae and Cysts in Faeces.*

These preliminary experiments were made by the old-fashioned method of introducing living amoebae (*per anum*) or cysts (*per os*) in the fresh faeces of a monkey into the alimentary canal of a kitten. This method involves various undesirable and unknown factors which nowadays can be eliminated: for the required amoebae and cysts in fresh faeces are invariably, in the case of a macaque, accompanied by other organisms (other species of amoebae, flagellates, etc.), so that a clean experiment is out of the question. In my own experiments, however, I ascertained with certainty that all faecal material used contained abundance of the organism (*E. histolytica*) which I desired to study, and I also determined accurately whether any other protozoa were—or might have been—present in it: for I not only carefully examined the material before inoculation, but I had also previously studied the entire protozoal fauna harboured by every monkey whose faeces I employed.

All these experiments gave entirely negative results. I fed 3 kittens (Nos. 1, 9, 10) on enormous numbers of cysts of *E. histolytica* concentrated from the faeces of one *M. sinicus* (Susanna): I injected 2 others (Nos. 3, 4) intrarectally with numerous active amoebae in the faeces of another *M. sinicus* (Polo): and I injected 2 others (Nos. 5, 6) twice apiece, on successive days, intrarectally with abundant amoebae from one *M. rhesus* (Jacko). The kittens were closely watched, and their faeces examined microscopically, for a time sufficiently long to determine the results of the experiments with certainty. One kitten (No. 6) died, and 3 others (Nos. 1, 5, 9) were ultimately killed. The *post-mortem* examinations yielded no evidence of amoebic infection. The remainder (Nos. 3, 4, 10) were finally discharged as normal and uninfected animals. The essential details of this series of experiments are shown in Table II (p. 16).

From these failures I drew the conclusion—which further work confirmed—that the strains of "*E. histolytica*" present in 3 of my monkeys (2 *M. sinicus* and 1 *M. rhesus*) were usually incapable of infecting normal kittens: for no kitten suffered from dysentery, and none showed any evidence of infection after the experiment.

[1] See p. 49 *infra*.

Table II.

Kitten No.	Experiment	Source of material	Date	Result
1	Fed with cysts	Faeces of *M. sinicus* (Susanna)	12. xi. 24	Negative. Killed 9. xii. 24. No amoebae or amoebic lesions found *post mortem*
3	Amoebae injected intrarectally	Faeces of *M. sinicus* (Polo)	10. xi. 24	Negative till 19. xii. 24, when used for another (negative) experiment. Finally discharged as normal 13. i. 25
4	Amoebae injected intrarectally	Faeces of *M. sinicus* (Polo)	10. xi. 24	Negative till 19. xii. 24, when used for another (negative) experiment. Finally discharged as normal 9. ii. 25
5	Amoebae injected intrarectally	Faeces of *M. rhesus* (Jacko)	25. xi. 24	Negative till 17. xii. 24, when used for another experiment. Finally killed 24. i. 25. No amoebae or amoebic lesions *post mortem*
	Repeated		26. xi. 24	
6	Amoebae injected intrarectally	Faeces of *M. rhesus* (Jacko)	25. xi. 24	Negative till 6. xii. 24, when used for another experiment—also negative. Died 25. i. 25. No amoebae or amoebic lesions *post mortem*. Enteritis and peritonitis, due to heavy natural infection with worms
	Repeated		26. xi. 24	
9	Fed with cysts	Faeces of *M. sinicus* (Susanna)	29. xii. 24	Negative till 23. i. 25, when killed. No amoebae or amoebic lesions *post mortem*
10	Fed with cysts	Faeces of *M. sinicus* (Susanna)	29. xii. 24	Negative till 23. i. 25, when used for another experiment. Finally discharged as negative and normal 4. iii. 25

These researches were begun before I had learnt how to cultivate *E. histolytica*. But I acquired this knowledge at the same time, and a second series of attempts was therefore made with amoebae and cysts from cultures.

(ii) *Indirect Attempts at Infection by means of Cultures.*

For this series of experiments I first isolated strains of *E. histolytica* in culture from 4 different monkeys (2 *M. rhesus* and 2 *M. sinicus*), and then attempted to infect kittens with them by injecting the culture-amoebae intrarectally or administering culture-cysts *per os*. These experiments were more satisfactory than those previously performed, because they were all made with "pure" cultures—that is, with cultures containing *E. histolytica* (with a mixed bacterial flora) but no other protozoa. Every culture used was carefully controlled by microscopic and cultural tests.

I fed 2 kittens (Nos. 3 and 4, already used unsuccessfully in the first series) on culture-cysts of *E. histolytica* (Strain *M.*4) from *M. rhesus* (Delilah) with completely negative results. I also fed 2 other kittens (Nos. 7, 8) on culture-cysts of another *rhesus*-strain (*J.*) from a different monkey (Jacko), and injected the culture-amoebae of this strain intrarectally into 2 others (Kittens 5 and 6—used unsuccessfully in the first series). All these kittens remained completely negative. None of them showed any sign of infection—

no amoebae being discoverable microscopically in their faeces, or in cultures made therefrom. No kitten suffered from dysentery, and those which ultimately died or were killed showed no amoebic lesions *post mortem*.

At the time when these experiments were made I discovered[1] that a strain of *E. histolytica* might lose its power of infecting kittens through prolonged cultivation. As all the strains here used had been previously cultivated for several weeks at least, it therefore seemed desirable to test a more freshly isolated strain. Accordingly, I isolated another strain (*S.*[2]) of *E. histolytica* from Susanna (*M. sinicus*)—whose parasites had given negative results in the first series of experiments—and tested it on a new kitten (No. 14) as soon as possible after isolation (4th serial subculture[2]). But the result was likewise entirely negative.

I therefore inferred that the strains of "*E. histolytica*" present in 4 of my monkeys (2 *M. rhesus* [Jacko and Delilah] and 2 *M. sinicus* [Susanna and Polo]) were incapable of infecting ordinary kittens. To me the evidence appeared definite and conclusive. But although these results were so discouraging, I did not accept them as final. I continued the experiments with amoebae from my other monkeys, and next investigated a strain (*B.*) which I isolated from another *M. sinicus* (Bonar). This strain behaved in a way which was quite unexpected.

Strain B.—This strain of *E. histolytica* was isolated from the normal faeces of Bonar (*M. sinicus*) on 13 March 1925. It grew excellently; and after daily transplantation for several days, the 5th serial subculture was found to be pure (*i.e.* free from all other protozoa) and was injected intrarectally into Kitten 15, which had never been used for any previous experiments. (The culture used was made on 17. iii. 25 and injected on 19. iii. 25.) The day after inoculation (20. iii. 25) Kitten 15 passed blood and mucus with its faeces, and in this material *E. histolytica* was easily discoverable. Cultures made from the bloody mucus showed abundant amoebae, and there could thus be no doubt that Kitten 15 had contracted amoebic dysentery almost immediately after injection with Strain *B.* Subsequent observations fully confirmed the diagnosis.

The whole of this series of experiments is summarized in Table III (p. 18). From this it will be seen that 4 pure strains of *E. histolytica* (*M.*4, *J.*, *S.*[2], *B.*) isolated from 4 different monkeys (2 *M. rhesus* and 2 *M. sinicus*) were tested on 8 kittens. Both strains from *M. rhesus* gave entirely negative results: one *sinicus*-strain (*S.*[2]) likewise proved incapable of infecting a kitten, but the other (*B.*) infected promptly and produced typical amoebic dysentery.

I must add here that all strains of *E. histolytica macacorum* employed in this series of experiments were morphologically indistinguishable from one another. Alive and in fixed and stained preparations they appeared exactly

[1] See Dobell and Laidlaw (1926*a*).

[2] It is usually impossible to test any strain earlier, as it takes several days at least to separate the amoebae from concomitant protozoa and prove their purity.

Table III.

Kitten No.	Date and experiment	Strain of amoebae used*	Origin of strain	Strain isolated	Result
3	19. xii. 24. Fed with culture-cysts	*M.*4 (7)	*M. rhesus* (Delilah)	15. xi. 24	Negative. Discharged as normal 13. i. 25
4	19. xii. 24. Fed with culture-cysts	*M.*4 (7)	*M. rhesus* (Delilah)	15. xi. 24	Negative. Discharged as normal 9. ii. 25
5	17. xii. 24. Injected intrarectally with culture-amoebae	*J.* (12)	*M. rhesus* (Jacko)	24. xi. 24	Negative till 24. i. 25, when killed. No amoebae or amoebic lesions found *post mortem*
6	6. xii. 24. Injected intrarectally with culture-amoebae	*J.* (10)	*M. rhesus* (Jacko)	24. xi. 24	Negative till 25. i. 25, when found dead. No amoebae or amoebic lesions *post mortem*
7	17. xii. 24. Fed with culture-cysts	*J.* (11)	*M. rhesus* (Jacko)	24. xi. 24	Negative till 4. i. 25, when killed. No amoebae or amoebic lesions found *post mortem*
8	17. xii. 24. Fed with culture-cysts	*J.* (10)	*M. rhesus* (Jacko)	24. xi. 24	Negative till 27. xii. 24, when found dead. No amoebae or amoebic lesions *post mortem*
14	3. iii. 25. Injected with culture-amoebae intrarectally	*S.*[2] (4)	*M. sinicus* (Susanna)	20. ii. 25	Negative till 16. iv. 25, when discharged as normal
15	19. iii. 25. Injected with culture-amoebae intrarectally	*B.* (5)	*M. sinicus* (Bonar)	13. iii. 25	*Positive.* Amoebic dysentery 20. iii. 25

* The figure in parenthesis denotes the serial number of the subculture used.

alike. But in cultures they displayed individual physiological differences which were, for the most part, undoubtedly referable to diversities in their concomitant bacterial floras. None of the monkeys from which these strains were cultivated suffered from dysentery during the period of observation: they were all normal and healthy in every way. Moreover, all the strains (including Strain *B.*, which proved to be pathogenic to kittens) ingested bacteria in their original hosts and in cultures, and all of them refused to ingest human red blood-corpuscles when tested *in vitro*[1].

The negative experiments made in this series were even more conclusive than those made in the first: for I was now able to check all microscopic findings by means of cultures, and I therefore have every confidence in the results recorded.

From all my work up to this point I concluded that most strains of *E. histolytica* living in my macaques were incapable of infecting normal kittens; but that one strain at least (from *M. sinicus*) could do so, and thereby cause amoebic dysentery. My one successful experiment with Strain *B.* (from Bonar) formed the starting-point of a further series, which must now be described.

[1] This subject is discussed in greater detail in a later section (p. 55).

(iii) *Further Experiments with Strain* B.

Strain *B.* (*E. histolytica macacorum*) was obtained from Bonar (*M. sinicus*) on 13 March 1925, and voluntarily[1] abandoned 5 weeks later at the 23rd serial subculture (17 April 1925). It was grown throughout in a medium composed of inspissated horse-serum covered with Ringer-eggwhite, and containing solid rice-starch (HSre + S; Dobell and Laidlaw, 1926 *a*). When I found that this strain readily infected Kitten 15, I resolved to investigate it further; and such experiments as I was able to perform will now be recorded.

Kitten 15 (experimentally infected with Strain *B.* on 19. iii. 25) was kept under close observation, and continued to suffer from amoebic dysentery for a considerable period. The disease followed a course which must be called "chronic" rather than "acute." From the bloody mucus in this animal's dejecta I isolated altogether 8 substrains of *E. histolytica* in pure culture, at various dates, and studied 6 in detail.

On 25 April 1925 (36 days after onset of the disease) I injected some of the fresh bloody mucus—containing abundant amoebae—from Kitten 15 into Kitten 20, a normal healthy animal not used previously. Next day (26. iv. 25) this kitten likewise passed blood, mucus, and numerous active amoebae with its faeces; and afterwards it suffered continuously from amoebic dysentery until it was sacrificed a month later. This experiment showed that the amoebae in Kitten 15 were directly transmissible to other kittens.

Strain *B.*, which had meantime been kept under cultivation *in vitro*, was tested a second time on 14 April 1925 (21st serial subculture, 32 days after isolation of the strain), when 2 new kittens (Nos. 17 and 18) were inoculated. Each received all the amoebae in a single culture intrarectally. Both, as a result, contracted amoebic dysentery a few days afterwards, with typical amoebae (which were carefully studied and cultivated) in their discharges.

On 17 April 1925 (5 weeks after isolation) I made a final experiment with Strain *B.*, injecting the 23rd serial subculture intrarectally into Kitten 19. The result was again positive, the kitten developing amoebic dysentery like the others. This experiment proved that the strain remained infective to kittens for as long as it was under cultivation (35 days).

A further experiment was made to ascertain whether the substrains of *E. histolytica* (Strain *B.*) recovered in cultures from the infected kittens were transmissible to other kittens. I had abundant material for such experiments at the time, but selected for trial a specially well-growing substrain from Kitten 18 (infected on 14. iv. 25). This substrain (*K.*[2]18) was isolated on 24. iv. 25, and the 4th serial subculture was injected intrarectally into Kitten 21 on 30. iv. 25 (a 3-day culture containing enormous numbers of *E. histolytica*). The kitten contracted amoebic dysentery 2 days later, and suffered from it for the rest of its life.

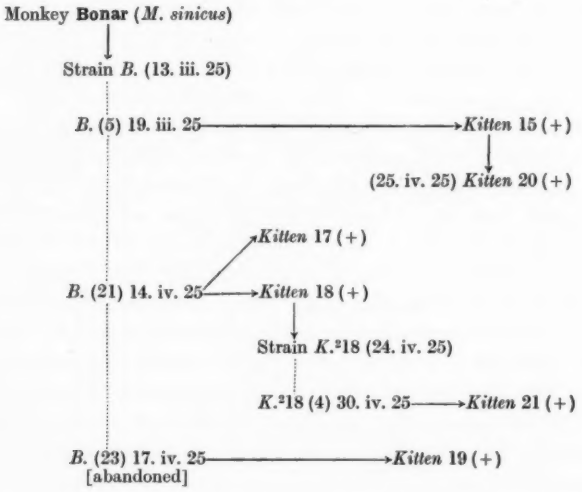
[1] This strain I could have propagated further without difficulty: but at the time I had so many other strains under cultivation, and so many other experiments in hand, that I was actually forced to sacrifice Strain *B.* against my will.

This experiment proved that substrains of amoebae cultivated from kittens experimentally infected with Strain *B.* were infective for other kittens. I have no doubt that experiments of the same character could have been continued indefinitely: for with one notable exception—to be described presently—all kittens directly inoculated with Strain *B.*, or with amoebae in (or culturally derived from) kittens experimentally infected with this strain, contracted amoebic dysentery.

This series thus formed a striking contrast to those previously performed; for whereas the earlier series, though conducted with equal care, gave nothing but negative results, the experiments with Strain *B.* were almost invariably positive.

All the positive experiments with Strain *B.*—just described—are summarized graphically in the accompanying Scheme (A).

Scheme A.


Monkey Bonar (M. sinicus)
Strain B. (13. iii. 25)
B. (5) 19. iii. 25
Kitten 15 (+)
(25. iv. 25) Kitten 20 (+)
Kitten 17 (+)
B. (21) 14. iv. 25
Kitten 18 (+)
Strain K.²18 (24. iv. 25)
K.²18 (4) 30. iv. 25
Kitten 21 (+)
B. (23) 17. iv. 25
[abandoned]
Kitten 19 (+)

As will be evident, I successfully infected 6 kittens altogether (Nos. 15, 17, 18, 19, 20, 21) in various ways with *E. histolytica* (Strain *B.*) from *M. sinicus*. These kittens were very carefully studied, and afforded the following additional information:

Course of the Disease.—The most striking feature of all these infections—as compared with similar infections with strains of *E. histolytica* from Man—was their mildness. All the kittens used were healthy, well-nourished, and well-tended animals, and they seemed at first to suffer little or no inconvenience from their dysentery. Although they passed with their faeces, at times, much blood and mucus containing abundant active amoebae, they did not display any other serious symptoms. They had good appetites, in general, and most

of them remained lively and playful until they were finally killed. Strain *B.* appeared, in fact, to cause in kittens an infection similar to the subacute or chronic type—rather than the acute—commonly seen in human beings. The superacute or fulminating dysentery so often seen in kittens parasitized by human strains of *E. histolytica* was never produced.

I sacrificed 5 of the 6 kittens, after they had suffered from this "chronic" dysentery for various periods, in order to obtain a good series of stages for histological study. Kitten 17 was killed on the 14th day of the disease, Kitten 21 on the 18th, Kitten 18 on the 23rd, Kitten 20 on the 28th, and Kitten 19 on the 35th. At the time of death all these animals were in quite good general condition, though heavily infected. I have no doubt that they could all have been kept alive much longer.

In order to ascertain how long a kitten infected with Strain *B.* could survive, and to study the course of the infection in greater detail, I did not sacrifice the first-infected kitten (No. 15) at an early stage, but kept it under observation for a longer period. My observations on this animal must now be described.

The Case of Kitten 15.—This little cat (a pretty tabby female) was born in my animal-room on Christmas Eve, 1924, and was under my daily personal observation for the whole of its life. It was originally a normal, strong, and very healthy animal, but always rather under-sized. It was not inoculated with Strain *B.* until it was nearly 3 months old: nevertheless, as already noted, it became infected and contracted amoebic dysentery almost immediately. It was inoculated at 2.45 p.m. on 19 March 1925, and afterwards passed no faeces until the following day. At 11.15 a.m. on 20. iii. 25 (20½ hours after the injection), however, it evacuated a large mass of solid faeces followed by a few drops of bloody mucus. This material was examined at once, and found to contain amoebae (*E. histolytica*): it was also inoculated into a culture-tube, and produced a very good growth of the same parasites—which were subcultivated and studied further. Afterwards this kitten continued to pass blood, mucus, and amoebae—of which other substrains were isolated and investigated—for the rest of its life (10 weeks).

I kept a detailed record of this animal, but it is too long to reproduce here in full. The chief features of interest are shown in Table IV, in which I have entered the results of my observations, day by day, from 20 March 1925, when the kitten was first found to be infected, until 26 May 1925 when it was killed. The number of stools passed *per diem* throughout is here recorded, together with the results of their microscopic and cultural examination. (Under the heading "Amoebae," the sign + indicates that the evacuations were examined microscopically, and *E. histolytica* was found: the sign − means that an examination was made, but no amoebae discovered: 0 denotes no examination made. Under "Cultures," similarly, + indicates a positive culture, − a negative culture, and 0 no culture made.)

Table IV.

Kitten 15 (inoculated 19. iii. 25) *Anno* 1925

Date (Mar.–Apr.)	20	21	22	23	24	25	26	27	28	29	30	31	1	2	3	4	5	6
Stools	1	1	2	2	1	1	1	1	1	1	1	2	1	2	2	0	2	2
Amoebae ...	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+	+
Cultures ...	+	0	0	+	0	0	0	0	0	0	0	+	0	0	0	0	0	0

Date (Apr.)	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Stools	2	2	2	2	3	3	4	1	3	3	1	1	2	4	1	1	3	2
Amoebae ...	+	+	+	+	+	+	–	0	+	+	0	0	+	+	0	0	+	+
Cultures ...	0	0	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0

Date (Apr.–May)	25	26	27	28	29	30	1	2	3	4	5	6	7	8	9	10	11	12
Stools	4	1	1	3	4	3	2	2	1	1	3	3	2	2	1	3	1	1
Amoebae ...	+	0	0	+	0	0	0	0	+	+	+	0	0	+	+	0	0	0
Cultures ...	+	0	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0

Date (May)	13	14	15	16	17	18	19	20	21	22	23	24	25	26
Stools	2	0	2	3	2	1	2	2	2	1	0	2	1	2
Amoebae ...	0	0	0	0	0	0	+	0	0	–	0	+	+	+
Cultures ...	0	0	0	0	0	0	0	0	0	0	0	+	0	+

From this table it will be seen that the number of stools passed *per diem* was never great—the maximum being 4 (on only four occasions). As a rule blood, mucus, and numerous amoebae were present; but on several days little or no blood was seen, and the stools were solid and formed. On two days (April 13 and May 22) no amoebae at all could be found, and several times the general condition of the kitten led me to think that it might recover. However, the disease progressed steadily, with intermissions, for 9 weeks. In the 10th week the kitten was obviously weaker. It was becoming emaciated, had lost its appetite, and ceased to clean itself. Its stools were purulent, and contained fewer amoebae—indicating a secondary bacterial infection of the large gut. As I had every reason to believe that this animal could not live much longer, I chloroformed it on 26 May 1925—69 days after it was originally injected. It was then just 5 months old: but as it ceased to grow after it became infected, and towards the end lost weight rather rapidly, it weighed only 540 grammes on the day of its death. During the whole course of its disease—until the last day—it never seemed seriously ill, and never at any time appeared to be in pain.

Post-mortem Findings.—All the kittens used for these experiments were dissected immediately after death, and their intestines preserved for histological study. I have not yet had time to section and examine all this material, however, so will now give only a brief account of the macroscopic findings[1].

All six kittens showed typical lesions in the large intestine, and none showed any signs of secondary amoebiasis (liver abscess, etc.). The ulceration and erosion of the intestinal mucosa varied in extent in different animals, but

[1] The detailed microscopic findings from this series of experiments, and an account of the pathological changes produced in kittens by Strain *B.*, will be reported later.

differed in no way from what is commonly seen in kittens infected with human strains of *E. histolytica*. As was to be expected, Kitten 15 showed the most extensive lesions. The whole of its large intestine was eroded internally, and appeared gangrenous. The contents consisted of faecal material and muco-pus —not obviously bloody—in which there were numerous amoebae at every level. In this animal there was a secondary bacterial infection of the gut wall, while the other kittens—killed at earlier stages of the disease—showed typical uncomplicated amoebic ulceration. Although the mesenteric glands were enlarged in all the kittens there was never any notable thickening of the intestinal wall—not even in Kitten 15. The outward appearance of the large intestine, on first opening the belly, was almost normal in all animals. Kitten 17 (killed 14 days after infection) showed macroscopically only small lesions in the region of the ileo-colic juncture: it had no conspicuous ulcers in the rest of the colon. Kitten 21 (killed on the 18th day) and Kitten 18 (killed on the 23rd day) showed similarly slight ulceration. But in Kittens 20 and 19 (killed on the 28th and 35th days respectively) the pathological changes were more extensive and severe.

The *post-mortem* findings, therefore, so far as they have yet been investigated, are consistent with the clinical and protozoological diagnoses. Strain *B.* produced amoebic dysentery in all infected kittens, but it was of a subacute or chronic and slowly progressing type conspicuously different from the acute and rapidly fatal dysentery generally produced by human strains of *E. histolytica*.

Latent Period.—Kitten 15 acquired its infection, and began to suffer from dysentery, almost immediately after inoculation: but this was not the case with all the other kittens. After inoculation, the dejecta of every animal were examined daily—both microscopically and culturally—in order to determine the "incubation period" as exactly as possible. On several occasions amoebae were recovered in cultures from the faeces a day earlier than they were found by direct microscopic examination: but taking the first day (after inoculation) on which amoebae were discovered in the stools, by either method, as marking the beginning of the disease, the "latent," "prepatent," or "incubation" period for each of the six kittens studied was as follows:

Kitten	15	first showed	infection	on the	1st	day after injection
,,	17	,,	,,	6th	,,	,,
,,	18	,,	,,	3rd	,,	,,
,,	19	,,	,,	9th	,,	,,
,,	20	,,	,,	1st	,,	,,
,,	21	,,	,,	2nd	,,	,,

This therefore gives a minimum "incubation period" of 1 day, and a maximum of 9 days—the average being roughly 3½ days for the whole series. The series is obviously too small to warrant generalization, but the figures show that Strain *B.* usually established itself in kittens soon after introduction.

So far I have described only my positive experiments with Strain *B*. But I also made some which were negative, and these must now be recorded. They were all made upon the same kitten—No. 16.

The Case of Kitten 16.—My experiments with this kitten showed that it was entirely refractory to infection with *E. histolytica macacorum* (Strain *B*.). As it was important to establish the fact that natural resistance to infection may occur, I studied this animal in detail, and will now briefly recapitulate its history.

Kitten 16 was an ugly and irregularly marked black-and-white female, which was characterized by its extraordinary liveliness and vigour[1]. When it was about 8 weeks old (on 31 March 1925) I inoculated it intrarectally with bloody mucus containing abundant active *E. histolytica* from the dejecta of Kitten 15. But no infection followed. Kitten 16 remained superactive and healthy until 9 April 1925, when I reinoculated it with a massive dose of *E. histolytica* from cultures. The cultures used for this experiment were derived from the material used for the first attempt. When I first injected Kitten 16 with amoebae from Kitten 15, I also incubated a little of the inoculum in a culture-tube (to make sure that the amoebae were healthy), and propagated the strain so initiated (*BK*.[3]) further. It was an excellent strain, and all the amoebae in two rich subcultures (the 4th and 5th) were mixed together and injected into Kitten 16 at the second attempt. Again, however, no infection resulted. The kitten remained extraordinarily lively and well, developed no symptoms of dysentery, and never discharged any amoebae in its evacuations.

As this experiment again gave completely negative results after a week's careful work, I finally injected all the amoebae from a rich culture of Strain *B*. (original strain, 23rd serial subculture) into Kitten 16 on 17 April 1925. This likewise had no effect. The kitten continued to grow, remained normal and violently active—developing no dysentery, and passing no amoebae with its faeces—for a whole month, during which time I examined it repeatedly both microscopically and culturally. I then (15. v. 25) discharged it—a half-grown healthy cat—as uninfected and uninfectible.

In order to appreciate the cogency of these negative experiments, it is necessary to consider the parallel positive results obtained at the same time and already recorded. Each of the three negative experiments made on Kitten 16 had a carefully planned positive counterpart in another kitten[2], as is shown in the accompanying table (Table V).

[1] I have never seen any other kitten which displayed such astonishing vitality. It had a huge appetite and grew very rapidly. When let loose, it would dash about the room at express speed, run up the walls, and turn somersaults—apparently for the sheer joy of exercising itself: and when it was allowed to play with other kittens, it used to leap round them, run under them, knock them down, and throw them about until they were terrified. It played all manner of games with me, and was never so happy as when it was being chased. Yet it was docile and good-tempered, and seldom bit or scratched, though all its reactions were supernormal and its playfulness almost pathological.

[2] These positive results are already exhibited in Scheme A (p. 20).

Kitten 16 Table V. *Anno* 1925

Experiment	Result
(1) Intrarectally injected with amoebae from *Kitten* 15 (31. iii. 25)	Negative
[Parallel experiment with *Kitten* 20 (25. iv. 25)	*Positive*]
(2) Intrarectally injected with culture-amoebae derived from infected kitten (9. iv. 25)	Negative
[Parallel experiment with *Kitten* 21 (30. iv. 25)	*Positive*]
(3) Intrarectally injected with culture-amoebae, Strain *B.*, 23rd subculture (17. iv. 25)	Negative
[Parallel experiment with *Kitten* 19 (17. iv. 25)	*Positive*]

The negative results obtained with Kitten 16 were controlled with every care. Whilst this animal was under observation I inspected all its dejecta every day; I examined them microscopically on 25 occasions; and I made 10 attempts to recover *E. histolytica* from suitable specimens in culture[1]. I am fully satisfied, consequently, that this kitten was incapable of being infected with Strain *B.*

(iv) *Further Attempts with Cysts in Faeces and Amoebae in Cultures.*

When I discovered that Strain *B.*, isolated from my monkey Bonar (*M. sinicus*), was the only one capable of infecting kittens, I resolved to make some further experiments with the cysts in this monkey's faeces. I was able to make only a few, but the results were interesting.

On 12 September 1926 I concentrated the *E. histolytica* cysts—which were very numerous—in Bonar's faeces (by centrifuging and sedimentation in sterile distilled water[2]), and after treating them with dilute hydrochloric acid (to kill off concomitant amoebae[3]) I fed half the concentrate immediately to a kitten (No. 28), and the other half 4 days later to its sister (Kitten 31). These kittens were fine healthy animals born in my animal-room on 1 August 1926, and therefore approximately 6 weeks old at the time. They had both been under my daily observation from birth, and had never been used previously for any other experiments.

The cysts fed to Kitten 28 were 5 hours old (after leaving the monkey's gut), while those fed to Kitten 31 had been kept at room-temperature for 4 days. But both kittens contracted amoebic dysentery on the 6th day after the infective feed—No. 28 on September 18, No. 31 on September 22. On these dates respectively they first passed bloody mucus, containing *E. histolytica*, with their faeces; and afterwards they suffered for a short period from a mild form of amoebic dysentery, from which they both recovered spontaneously. Their histories must now be considered separately.

[1] More attempts were not made because the kitten latterly passed—at infrequent intervals—faeces so hard that it seemed a waste of time to look for amoebae in them.

[2] Previous experiments had proved that such treatment was not injurious to cysts.

[3] See Dobell and Laidlaw (1926*a*), Dobell (1927). After treatment with acid, a small sample of these cysts was inoculated into a culture-tube and incubated. It gave an excellent culture—proving that the cysts were viable.

The Case of Kitten 28.—This kitten's infection has already been partly described elsewhere, because it furnished me with a strain of *E. histolytica* (*K*.28 c.) whose life-history *in vitro* I studied in detail[1].

Kitten 28, a tabby male, was fed with cysts (5 hours old) at 5 p.m. on 12. ix. 26. It passed a normal stool half-an-hour later. During the next 3 days (September 13, 14, 15) it passed no faeces at all, but remained otherwise normal. On 16. ix. 26 it voided some normal faeces, containing no blood, mucus, or amoebae: on 17. ix. 26 again no faeces were passed. Next day (18. ix. 26), however, it evacuated early in the morning a stool consisting partly of solid normal faeces and partly of semi-liquid material containing mucus but no macroscopically visible blood. In this material amoebae were found on microscopic examination, and in a culture made from it an abundant growth of *E. histolytica* was obtained. On September 19, and for 5 days following, the kitten passed 1 or 2 stools containing bloody mucus and abundant amoebae each day. The dysentery then unexpectedly cleared up until September 30, when a relapse occurred. On this date 3 stools were evacuated. No blood was seen in the dejecta, though excess of mucus was present; but in this mucus amoebae were found by microscopic examination, and from it they were recovered in culture.

From 1 October 1926 onwards, Kitten 28 never suffered from dysentery. Its stools rapidly became normal, and no amoebae were discoverable in them either microscopically or culturally. I made careful microscopic examinations of its faeces on 12 occasions, and attempted to cultivate amoebae from them on 9: but all results were completely negative until November 10. It was then a normal, healthy, actively growing cat, which had apparently recovered completely from its infection. My observations on this animal from the date of infection (12. ix. 26) to the end of the experiment (10. xi. 26) are summarized in Table VI (p. 27).

In order to make sure that Kitten 28 had really recovered from his experimental infection with *E. histolytica*, I gave him—after careful study for 6 weeks, with entirely negative results—to good friends who have since kept him as a pet, and who have kindly allowed me to see him from time to time during the last four years. I can therefore state, from personal knowledge, that "Kitten 28" is now a full-grown cat, in first-rate physical condition, and has never shown any signs of amoebic infection since I discharged him as "negative" on 10 November 1926.

That Kitten 28 was once infected with *E. histolytica* I can prove conclusively: for I still have under cultivation a strain (*K*.28 c.) of this parasite isolated from his dejecta on 18 September 1926, with a full record of its history. The kitten itself—now a beautiful adult cat—is, at the moment of writing (1930), a living proof that he recovered from his experimental infection.

Attempts to transmit Strain K.28 c.—I made seven unsuccessful attempts to infect other kittens with Strain *K*.28 c., isolated from Kitten 28 when it

[1] See Dobell (1928), p. 367.

was suffering from amoebic dysentery. This strain not only caused very mild dysentery in the originally infected kitten (No. 28) but also proved to be non-transmissible. All these negative experiments were very carefully controlled—all kittens' droppings being inspected daily throughout, and frequent micro-

Table VI[1].

Kitten 28 (fed 12. ix. 26) *Anno* 1926

Date (Sept.) ...	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
Stools	1	0	0	0	1	0	1	1	2	1	2	2	1	0	0
Amoebae ...	0	0	0	0	–	0	+	+	+	+	+	+	+	0	0
Cultures	0	0	0	0	0	0	+	0	0	0	–	0	0	0	0
Date (Sept.–Oct.)	27	28	29	30	1	2	3	4	5	6	7	8	9	10	11
Stools	1	1	0	3	2	2	1	0	1	1	2	1	0	1	1
Amoebae ...	–	–	0	+	–	–	–	0	–	–	–	0	0	0	0
Cultures	–	–	0	+	–	–	–	0	0	0	0	0	0	0	0
Date (Oct.) ...	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
Stools	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1
Amoebae ...	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cultures	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Date (Oct.–Nov.)	27	28	29	30	31	1	2	3	4	5	6	7	8	9	10
Stools	1	1	1	1	1	1	1	1	2	2	1	1	1	1	1
Amoebae ...	0	0	0	–	0	0	0	–	–	–	0	–	0	–	0
Cultures	0	0	0	–	0	0	0	–	–	–	0	–	0	–	0

Table VII. *Attempts to infect kittens with Strain K.*28c. (isolated from Kitten 28, 18. ix. 26).[2]

Kitten No.	Experiment	Date	Serial No. of subculture used	Result
32	Fed with culture-cysts	26. ix. 26	1	Negative. Discharged as normal 12. xi. 26
30	Injected intrarectally with amoebae	7. x. 26	11	Negative. Discharged as normal 12. xi. 26
33	Fed with culture-cysts	26. i. 27	72	Negative. Discharged as normal 2. iii. 27
38	Injected intrarectally with amoebae	17. iii. 27	80	Negative. Discharged as normal 21. iv. 27
35	Fed with culture-cysts	11. iii. 27	93	Negative. Discharged as normal 21. iv. 27 [Still alive and well.]
37	Fed with culture-cysts	15. iii. 27	95	Negative. Discharged as normal 6. v. 27.
36	Injected intrarectally with amoebae	11. iii. 27	96	Negative. Killed 4.v.27. Normal and uninfected

[1] In this Table, under "Stools" the symbol 0 means "no faeces passed." Under "Amoebae" and "Cultures" 0 means "no microscopic examination (or culture) made": while the sign – indicates that a microscopic or cultural examination was made, but no amoebae found. The sign + means that amoebae were present in the stools, or in cultures made therefrom.

[2] The life-history of this strain *in vitro* has already been described elsewhere (Dobell, 1928). Apparent discrepancies in dates and subculture-numbers in the Table are due to the circumstance that several different lines of Strain *K.*28c. were tested in these experiments. As these lines were not transplanted simultaneously, serial subcultures of like number were frequently made on different dates.

scopic and cultural examinations[1] of them being made. Although I spent much time and work on these attempts, it seems unnecessary to record my findings in detail here. I therefore merely summarize the most important data in tabular form (Table VII, p. 27). The experiments were continued for a time sufficient to convince me, at least, that Strain *K*.28 c. (*E. histolytica macacorum*)—though derived from an undoubtedly infected kitten—was incapable of infecting other kittens. Its feeble infectivity is also attested by the fact that Kitten 28 recovered from the infection spontaneously.

These experiments may be contrasted with those previously shown in Scheme A (p. 20). It should be remembered that Strain *B.* and Strain *K*.28 c. were both derived originally from the same monkey—Bonar (*M. sinicus*).

The Case of Kitten 31.—As already mentioned, this kitten (a tabby female) was one of the same litter as No. 28. It was fed on concentrated *E. histolytica* cysts from Bonar's faeces on 16 September 1926 (4 days after they were passed). A small sample of these cysts, inoculated into a culture-tube and incubated at the same time, gave a good culture of amoebae. The kitten, like No. 28, first passed blood, mucus, and amoebae with its faeces 6 days after the infective feed (on September 22): and thereafter its history was similar.

Kitten 31, however, suffered from even milder dysentery than Kitten 28. It never passed much bloody mucus, and amoebae were discoverable in its dejecta for only 4 days (till September 25), and always in small numbers. Afterwards the dysentery gradually cleared up completely; though a little blood, in which no amoebae could be found either microscopically or culturally, was present in its stools occasionally for several weeks. Apart from this the kitten made an uninterrupted recovery, and was discharged as normal and uninfected on 12 November 1926. Between 25. ix. 26 (when amoebae were last found) and 12. xi. 26 I made repeated microscopic examinations of its faeces, and on 13 occasions I also attempted to recover *E. histolytica* from them in cultures. But all attempts were absolutely negative.

In order to follow this kitten further, I sent it—after the experiment just recorded—to the Farm belonging to the Institute, where it remained at large and grew into a normal healthy cat. But in the summer of 1928 I heard that it had strayed and was lost, so I can add no further details of its later history. I feel confident, however, that this animal made a complete spontaneous recovery from its infection with *E. histolytica*.

At a later date I made two further attempts to infect kittens with cysts from Bonar's faeces—administered *per os* in a similar way—but they were both negative. Kitten 36 was fed with concentrated cysts (3 days old) on

[1] Although all these kittens showed no symptoms and continued to pass normal faeces in which no amoebae were discoverable by direct microscopic examination, I made and examined cultures from them at intervals as an additional control. At least 6 negative cultures—usually more—were made from every animal in this series.

30 March 1927, and Kitten 37 with a similar concentrate (6 days old) on 2 April 1927. Both animals remained perfectly normal for the next 5 weeks —all microscopic and cultural examinations being completely negative for *E. histolytica*, and neither kitten displaying any dysenteric symptoms. I then killed No. 36, and found no amoebae or amoebic lesions *post mortem*. No. 37 I gave to a colleague, who took it home and kept it as a pet. It grew into a fine healthy cat, which was unfortunately killed in an accident[1] two years later.

The results of my four attempts to infect kittens *per os* with cysts of *E. histolytica* from the faeces of Bonar (*M. sinicus*) are summarized in Table VIII.

Table VIII. *Cysts of* E. histolytica *from faeces of* M. sinicus (*Bonar*) *fed to kittens.*

Kitten No.	Date of experiment	Age of cysts	Result
28	12. ix. 26	5 hours	*Positive.* Amoebic dysentery 18. ix. 26. Recovered spontaneously. Still alive and well [July 1930]
31	16. ix. 26	4 days	*Positive.* Amoebic dysentery 22. ix. 26. Recovered spontaneously. Normal for 2 years, when lost
36*	30. iii. 27	3 days	Negative till 4. v. 27, when killed. No amoebae or amoebic lesions found *post mortem*
37*	2. iv. 27	6 days	Negative. Discharged as normal 6. v. 27. Remained normal for 2 years, when killed in accident

* Kittens used previously for (negative) experiments with Strain *K*.28c. (See Table VII, p. 27.)

The negative results obtained in the last two experiments (Kittens 36 and 37) were not surprising: for it is notoriously more difficult to infect kittens by means of *E. histolytica* cysts administered *per os* than by amoebae injected *per anum*. Moreover, at the time when these later experiments were made, Bonar himself was possibly infected with more than one strain of the parasite: for he had been in frequent contact with all my other monkeys—whose strains were not infective for kittens—and I am therefore unable to prove that these two later experiments were carried out with the infective strain originally present in him. It is not unlikely that the cysts used latterly were those of uninfective strains which he had picked up from his companions. As I was unable to determine this point, I can only record my inconclusive experiments for what they may be worth.

I may also record here two other experiments made with other strains of *E. histolytica* which I isolated from Bonar (*M. sinicus*). It will be recalled that Dr Laidlaw and I recorded some years ago that a strain of *E. histolytica hominis* (*D.*) lost its infectivity for kittens on continued cultivation[2]. I found

[1] The owner informed me (in 1929) that the cat fell through the roof of his greenhouse, and was so badly cut by broken glass that he had to destroy it.

[2] Dobell and Laidlaw (1926*a*), p. 314.

in the foregoing experiments, however, that Strain *B.* retained its power of infecting kittens for as long as it was cultivated (5 weeks)[1]. I therefore tried to discover how long the infective amoebae in Bonar could retain their infectivity *in vitro*.

(*a*) On 6 August 1926 I initiated another strain (*BX.*) from *E. histolytica* cysts passed by Bonar six days previously (31. vii. 26). I cultivated and tested this strain carefully until 13 September 1926, when I injected the 24th serial subculture intrarectally into Kitten 29 (6 weeks old, and used for no other experiments)[2]. But the kitten never contracted dysentery, and was finally discharged as normal two months later (12. xi. 26). During this period it was under continuous observation, and its faeces were repeatedly examined microscopically with negative results: I also attempted to cultivate *E. histolytica* from its dejecta on 14 different occasions without success.

(*b*) On 3 August 1926 I started another strain (*BC.*) from *E. histolytica* cysts passed by Bonar 24 days previously (10. vii. 26). This strain was originated in the course of experiments made to determine the viability of cysts kept in water at room-temperature[3]. As it grew excellently, I cultivated it further and obtained cysts from it again. These cysts I hatched successfully, and I then continued the strain from the resultant amoebae. On 14 September 1926 I injected the 12th serial subculture (amoebae) intrarectally into Kitten 30[4] (used for no other experiments), but without result. The kitten never suffered from dysentery and never passed microscopically discoverable amoebae with its faeces; and after I had made 8 negative attempts to cultivate *E. histolytica* from its dejecta, I discharged it as normal on 12 November 1926.

These two experiments indicated that Strains *BX.* and *BC.* were, at the time of testing, uninfective for normal kittens. But they were inconclusive as regards the point at issue—whether or no prolonged cultivation induces non-infectivity. I did not test either strain until it had been under cultivation for 5–6 weeks, when it was found incapable of infecting a kitten: but as I made no experiment earlier[5], I cannot determine whether they lost their infectivity on cultivation, or had been non-infective from the beginning.

Summary of the Foregoing Experiments (pp. 15–30).

Nine attempts to infect kittens directly with *E. histolytica macacorum* by means of amoebae (*per anum*) or cysts (*per os*) in the faeces of 2 *M. sinicus* (Polo and Susanna) and 1 *M. rhesus* (Jacko), were wholly unsuccessful. Four similar experiments with cysts (*per os*) from another *M. sinicus* (Bonar) gave 2 positive (Kittens 28 and 31) and 2 negative results.

[1] See Scheme A, p. 20.

[2] This kitten belonged to the same litter as Nos. 28 and 31, which were successfully infected (though they both recovered) by means of cysts from Bonar's faeces. Cf. Table VIII, p. 29.

[3] See Dobell (1927).

[4] Brother of Nos. 28, 29, and 31.

[5] I did not make any trials with these strains at an earlier date because it then appeared to me unnecessary—Strain *B.* from the same macaque having remained fully infective for 5 weeks.

Eight attempts to infect kittens by means of "pure" cultures of *E. histolytica* from 2 *M. rhesus* (Jacko and Delilah) and 2 *M. sinicus* (Susanna and Bonar), gave 7 negative results and 1 positive. The one success (Kitten 15) was again obtained with a strain derived from *M. sinicus* (Bonar). This strain (*B.*) remained infective for kittens for at least 5 weeks (when its cultivation was discontinued), and was found to be readily transmissible from kitten to kitten—either directly, or by the intermediation of cultures.

All kittens (8 in number) which contracted amoebic dysentery, as a consequence of infection with *E. histolytica* from Bonar (*M. sinicus*), suffered from a subacute or chronic form of the disease. Two of them (Nos. 28 and 31, both infected by means of faecal cysts *per os*) recovered spontaneously; while the one kitten (No. 15, intrarectally infected with culture-amoebae) in which the dysentery was allowed to run its course survived for 10 weeks.

One kitten (No. 16) was found to be uninfectible with Strain *B.* after three successive attempts, though parallel experiments with other kittens gave positive results every time.

One strain (*K.*28c.) of *E. histolytica macacorum*, recovered from an experimentally infected kitten (No. 28) and cultivated and studied ever since, has been found to be untransmissible to other kittens (7 experiments).

The latent ("incubation") period for the 6 kittens infected with Strain *B.* (amoebae injected intrarectally) varied from 1 to 9 days—average approximately 3½ days. Both kittens infected *per os* by means of natural cysts from the monkey which furnished this strain showed a latent period of 6 days.

The intestinal lesions produced in kittens by *E. histolytica macacorum* (Strain *B.*) were indistinguishable macroscopically from those caused by *E. histolytica hominis*, but no kitten acquired a liver abscess or any other secondary (extra-intestinal) amoebic infection.

2. Experimental Infection of Macaques with *E. histolytica macacorum*.

The experiments now to be described were undertaken with the object of discovering whether or not the various strains of *E. histolytica* which I isolated from Macaques were transmissible to others, of the same and of different species. All my strains of *E. histolytica macacorum* were morphologically typical in every way, but it was necessary to find out—before concluding that they were all identical—whether strains from one species of *Macacus* could inhabit another: for example, whether a strain of *E. histolytica* from *M. sinicus* could live in *M. rhesus*, and *vice versa*.

Before any such experiments could be attempted, a great deal of preliminary work was necessary. The requisite strains of amoebae had to be isolated and studied for considerable periods in order to prove their purity; and an infallible method for causing encystation *in vitro* at any desired moment had to be discovered. Moreover, as all my macaques were naturally infected with *E. histolytica*, I had also to discover a certain method of eradicating

their own infections before I could attempt to reinfect them with other strains. The methods ultimately adopted have already been considered[1], and therefore need no further discussion here.

(i) *Transmission from* sinicus *to* sinicus.

My first experiment was an attempt to transmit a pure strain of *E. histolytica* from one *M. sinicus* to another *M. sinicus* by means of cultures. It was obviously necessary, before proceeding further, to prove that this could be done: because no such experiment had been made previously, and nothing was known as to the infectivity—or non-infectivity—of cultivated strains of *E. histolytica* (either *macacorum* or *hominis*) for monkeys of any species[2].

For this experiment I used a very carefully studied strain of *E. histolytica* which has already been referred to many times—*K*.28c., derived from my monkey Bonar (*M. sinicus*). This strain was under complete control *in vitro*. I had worked out its life-history in detail; and I could—and still can—obtain any stage in its development at will[3]. Cysts in Bonar's faeces were fed to a kitten (No. 28), which contracted amoebic dysentery[4]: and Strain *K*.28c. was isolated from this kitten's dysenteric dejecta on 18 September 1926. It was undoubtedly a pure strain of *E. histolytica macacorum* from *M. sinicus*.

The monkey used for the experiment was Polo (*M. sinicus*). His natural infections with *E. histolytica* were removed by treatment with emetine, as described elsewhere[5]; and before any attempt was made to reinfect him, his faeces had been consistently negative (daily examinations, both microscopically and culturally) for 30 consecutive days. That he was uninfected at the time of the experiment I cannot doubt.

Polo was fed on all the very numerous cysts present in three excellent cultures of Strain *K*.28c. (91st serial subculture) on 9 April 1927. (The cultures were made on 6. iv. 27: removed from the incubator 2 days later: and the cysts concentrated, pooled, and kept at room-temperature for another day before administration.) The daily microscopic and cultural examinations of the monkey's faeces were continued during, and for some time after, the experimental feeding. For the first 2 days the faeces remained negative. But the culture made on the third day gave a good growth of *E. histolytica*, though the microscopic examination revealed no infection: and on the fourth day—and ever afterwards—both microscopic and cultural tests were positive. This experiment is summarized in the accompanying Table IX.

[1] See p. 5 *supra*, and Dobell with Bishop (1929).

[2] All that I knew, at that date, was the discouraging fact—elicited in earlier work—that culture-cysts of *E. histolytica macacorum* were usually incapable of causing infection in kittens; though I also knew that prolonged cultivation of infective strains (both *macacorum* and *hominis*) might render them non-infective to these animals.

[3] See Dobell (1928), p. 367. The strain is still under cultivation, and at the moment of writing (July, 1930) has reached its 330th serial subculture in nearly 4 years.

[4] See above, p. 26.

[5] See Dobell with Bishop (1929), p. 456; *ibid.* Table VII, p. 467.

Table IX.

Polo (*M. sinicus*) fed with culture-cysts (*K.*28 c.) derived from Bonar (*M. sinicus*). 9. iv. 27		Dates of Examinations (1927) Mar. 11–Apr. 9	10	11	12	13	14
E. histolytica	faeces	−	−	−	−	+	+
	cultures	−	−	−	+	+	+

Polo remained infected with *E. histolytica* for the rest of his life (15½ months) —until I killed him on 28 July 1928. I studied his experimental infection during this period, and investigated various substrains[1] isolated from his faeces on different dates. They all appeared to be identical, and were morphologically and culturally indistinguishable from Strain *K.*28 c. Polo himself remained healthy and vigorous throughout and never suffered from dysentery, though he harboured a strain of *E. histolytica* which was originally pathogenic to a kitten, and which was derived from a monkey of the same species (Bonar) who had previously been proved to contain a strain of the parasite capable of causing dysentery in the cat[2]. When Polo was finally sacrificed, I could find no amoebic lesions in his large intestine *post mortem*. He appeared perfectly healthy in every way.

This single experiment conclusively resolved several different uncertainties. It showed that a strain of *E. histolytica* from *M. sinicus* could be readily transmitted, by way of cultures, to another monkey of the same species: that a strain from *M. sinicus* could again infect *M. sinicus* after passage through a kitten: that a *sinicus*-strain could retain its infectivity for *M. sinicus*—even though it had lost its infectivity for kittens[3]—after cultivation for as long as 7 months: that culture-cysts of Strain *K.*28 c.—though unable to infect kittens[4]—were normal cysts of *E. histolytica*, capable of infecting a monkey of the same species as that from which the strain originated: and that one and the same strain of *E. histolytica* may be, and may remain *in vitro*, harmless to *M. sinicus* though initially pathogenic to kittens[5].

Another point which the experiment determined—though one not yet mentioned—was the following. Strain *K.*28 c., since its isolation in September 1926, had been cultivated continuously *in vitro* in egg-serum medium (Ehs, Dobell-Laidlaw), and made to encyst from time to time by the addition of solid rice-starch[6]. The cysts so obtained were repeatedly kept for various periods, then hatched, and the resultant substrains continued. I wished to determine whether the intermittent occurrence of encystation, in this way, in

[1] Especially one (*P.*) which will be mentioned again in a later section (p. 51).

[2] See the foregoing experiments with Strain *B.* (p. 19) and Kittens 28 and 31 (pp. 26 and 28).

[3] See p. 26 *supra*.

[4] See Table VII, p. 27.

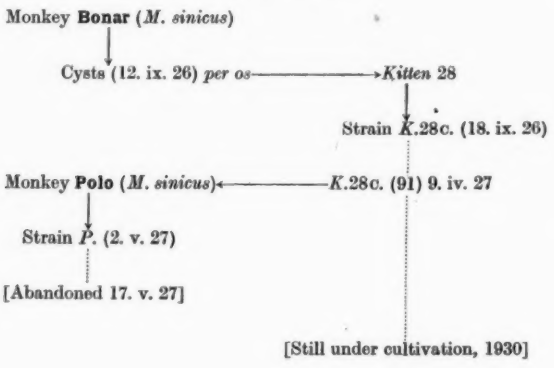
[5] The cysts from Bonar (who never suffered from dysentery) infected Kitten 28 and produced amoebic dysentery in it. But the strain (*K.*28 c.) isolated from this kitten produced neither dysentery nor intestinal lesions in Polo when the infection was experimentally established in him.

[6] See Dobell (1928), p. 370, and p. 5 *supra*.

the life of a strain, affected its infectivity: and the particular line of *K.*28c. used to infect Polo was one which had encysted more frequently, and had remained encysted for longer periods, than any other which I then possessed. At one stage in its history it had been continued from culture-cysts kept at room-temperature for 37 days—the maximum time for which I had then found that such cysts could live[1]. The experiment therefore showed me that a strain of *E. histolytica* intermittently allowed to remain encysted—even for considerable periods—in the course of cultivation, did not thereby lose its infectivity. This information has been of practical use in later work.

In order to exhibit the foregoing experiment briefly and graphically, I have summarized it in the accompanying Scheme B.

Scheme B.


Monkey **Bonar** (*M. sinicus*)
Cysts (12. ix. 26) *per os*
Kitten 28
Strain *K.*28c. (18. ix. 26)
Monkey **Polo** (*M. sinicus*)
*K.*28c. (91) 9. iv. 27
Strain *P.* (2. v. 27)
[Abandoned 17. v. 27]
[Still under cultivation, 1930]

(ii) *Transmission from* rhesus *to* sinicus.

My next experiment was to determine whether the *E. histolytica* naturally inhabiting one species of *Macacus* could be transmitted to another macaque of different species: and the attempt was made with a strain from *M. rhesus* which I tried to transmit by methods similar to those just described—but with slight variations (designed to afford additional information)—to *M. sinicus*.

The strain of *E. histolytica* used was one (*Jc.*) originally isolated[2] from the faeces of my monkey Jacko (*M. rhesus*) on 18 January 1928. I had previously isolated and studied numerous strains from this monkey, and unsuccessfully attempted to infect kittens with his amoebae[3]. Strain *Jc.* was morphologically indistinguishable from the others, and was a typical *E. histolytica macacorum*

[1] See Dobell (1927), p. 300. I have since found that if the cysts are kept in the ice-chest (8°–12° C.) they may occasionally survive for as long as 8 weeks (57 days).

[2] By Miss Ann Bishop—under my supervision—from material which I personally collected. The strain was originated for the purpose of other experiments (Laidlaw, Dobell, and Bishop, 1928); but as it grew excellently, and had been thoroughly tested, I resolved to keep it for further study. I am indebted to Miss Bishop for maintaining this strain for several months during 1928.

[3] Cf. Table II (p. 16) and Table III (p. 18).

in every way. I investigated it in detail, and satisfied myself that it was pure. After cultivation for 11 months, it was finally abandoned voluntarily on 21 December 1928.

Strain *Jc.* was propagated throughout in HSre + S medium (Dobell-Laidlaw), and cultivated continuously in the amoeboid state. It had been caused to encyst, and continued from the cysts so formed, only once in its history—at the very beginning, in order to make sure of its purity.

The monkey to which I tried to transmit this strain was Susanna (*M. sinicus*). I eradicated her natural *E. histolytica* infection by treatment with emetine, and took extreme precautions to prove that she was uninfected before I attempted the experiment of reinfection. Her emetine-treatment has already been described elsewhere[1]: and I will therefore merely note here that her faeces were consistently negative for *E. histolytica* for 70 consecutive days (microscopic and cultural examinations made every day, barring a break of 10 days at the end) before the present experiment was performed. During this period Susanna was in contact with only one other monkey (Rosa), who was treated and cured simultaneously. Every possible avenue of accidental reinfection or chance of relapse was—to the best of my belief—excluded absolutely.

Susanna was fed with a very large number of cysts from the 70th serial subculture[2] of Strain *Jc.* on 18 December 1928. For the next 2 days her faeces remained microscopically negative, but from the culture made on 20. xii. 28 I recovered *E. histolytica*. Three days after the experimental feeding, both microscopic and cultural examinations were positive: and the monkey then remained infected until 10 March 1929 (nearly 12 weeks), when I re-treated her with emetine and removed her infection.

The foregoing experiment is summarized in Table X.

Table X.

Susanna (*M. sinicus*) fed with culture-cysts (*Jc.*) derived from Jacko (*M. rhesus*). 18. xii. 28		Dates of Examinations (1928)					
		Oct. 10–Dec. 18	19	20	21	22	23
E. histolytica	faeces	–	–	–	+	+	+
	cultures	–	–	+	+	+	+

Susanna's infection was carefully studied, and several pure strains of *E. histolytica* were isolated from her faeces. One of these—Strain *JSc.*—was cultivated for 15 months and has been investigated in detail. Morphologically all strains recovered from Susanna after experimental reinfection with Strain *Jc.* have proved to be indistinguishable from this strain.

I must repeat here that Jacko himself, whilst under my observation (nearly 4 years), never suffered from dysentery; he showed no amoebic lesions in his

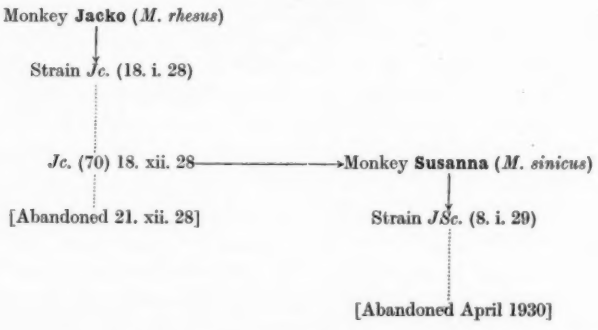
[1] See Dobell with Bishop (1929), p. 457; and *ibid.* Table VIII (p. 467).

[2] The cysts used for this experiment were derived from two cultures made on 13. xii. 28, and taken from the incubator on 15. xii. 28, and were kept at room-temperature for 3 days before they were fed to Susanna.

intestines *post mortem*; and his *E. histolytica* never infected kittens. Susanna likewise, who never displayed any dysenteric symptoms when parasitized by her own natural *E. histolytica* (which also failed to infect kittens), did not contract dysentery or evince any pathognomonic signs after she acquired Jacko's amoebae.

This experiment showed that a strain of *E. histolytica* derived from *M. rhesus* was readily transmissible, after continuous cultivation for 11 months, to *M. sinicus*. It is summarized graphically in Scheme C.

Scheme C.


Monkey **Jacko** (*M. rhesus*)
Strain *Jc.* (18. i. 28)
Jc. (70) 18. xii. 28
Monkey **Susanna** (*M. sinicus*)
[Abandoned 21. xii. 28]
Strain *JSc.* (8. i. 29)
[Abandoned April 1930]

(iii) *Transmission from* nemestrinus *to* rhesus, *and thence to* sinicus.

My third and last series of experiments in this connexion was similar to those just described, but more elaborate. The first series showed that a *sinicus*-strain could be easily transmitted to the same species: the second that a *rhesus*-strain was equally readily transmissible to *M. sinicus*. The present experiments were planned to test the infectivity of a strain of *E. histolytica macacorum* for three different species of *Macacus* (*nemestrinus*, *rhesus*, and *sinicus*).

On 3 August 1927 I isolated a strain (*Nc.*) of *E. histolytica* from the intestinal contents of a specimen of *Macacus nemestrinus* at necropsy[1]. This was an excellently growing strain—a morphologically typical *E. histolytica macacorum* in every respect. It was cultivated continuously in HSre + S medium (Dobell-Laidlaw) until 24 December 1928, when I abandoned it voluntarily. In the early stages of its history I caused it to encyst, and then continued it from these cysts (in the process of isolation and to test its purity): for the rest of the time it was propagated in the active amoeboid form[2].

In the middle of December 1928—after continuous cultivation for 16½ months—I again caused this strain to encyst, and fed the resultant cysts to a clean *M. rhesus*. These cysts were derived from two cultures (serial subculture

[1] See p. 10 and Table I (p. 8).

[2] I have to thank Miss Ann Bishop for maintaining this strain for me during several months in 1927 and 1928.

106, made on 13. xii. 28 and removed from the incubator on 15. xii. 28) containing very large numbers of typical ripe cysts of *E. histolytica*. They were kept at room-temperature for 3 days, and fed to the monkey on 18. xii. 28.

The monkey employed in this experiment was Rosa (*M. rhesus*), whose natural *E. histolytica* infections had been previously eradicated by treatment with emetine as elsewhere described[1]. Before she swallowed the cysts of Strain *Nc.* her faeces had been consistently negative for *E. histolytica* (careful daily examinations, both culturally and microscopically throughout, barring a break of 10 days at the end) during a period of 72 consecutive days. I am confident, therefore, that Rosa was not infected with *E. histolytica* at the time of the experiment.

For 2 days after the experimental feeding with culture-cysts of Strain *Nc.*, Rosa's faeces were consistently negative—microscopically and culturally. But the culture made on the third day was positive for *E. histolytica*—though the microscopic examination was negative—and on the fourth day both examinations were positive. Thereafter this monkey remained infected (*E. histolytica* being usually discoverable in the faeces microscopically, and recoverable in cultures) until 10 March 1929, when I eradicated the infection by a second treatment with emetine.

Whilst she was previously infected with her own original strains of *E. histolytica* (October 1926 to October 1928 = 2 years), Rosa never suffered from dysentery, nor displayed any symptoms referable to the infection. Afterwards, when reinfected (18. xii. 28) with Strain *Nc.* (from *M. nemestrinus*), she remained equally free from dysenteric symptoms until this infection was experimentally removed (10. iii. 29).

The foregoing experiment is summarized in Table XI.

Table XI.

Rosa (*M. rhesus*) fed with culture-cysts (*Nc.*) derived from *M. nemestrinus*. 18. xii. 28		Dates of Examinations (1928)					
		Oct. 8–Dec. 18	19	20	21	22	23
E. histolytica	faeces	–	–	–	–	+	+
	cultures	–	–	–	+	+	+

I isolated and studied several strains of *E. histolytica* from Rosa's faeces whilst she remained infected. One of these (*NRK.*) was specially studied; but all were closely similar and morphologically indistinguishable from Strain *Nc.*, from which they were derived.

Strain *NRK.* I isolated on 24 December 1928, and cultivated continuously in egg-serum medium without starch (Ehs, Dobell-Laidlaw: original Boeck-Drbohlav medium, slightly modified)—except when I desired to make it encyst. I finally relinquished it voluntarily, after careful study, in April 1930, when it was still growing abundantly and typically after 16 months' cultiva-

[1] See Dobell with Bishop (1929), p. 458; *ibid.* Table IX, p. 467.

tion. I used this strain for the next experiment—an attempt to transmit a *nemestrinus*-strain to *sinicus*, after passage through *rhesus*.

The monkey used for this experiment was Susanna (*M. sinicus*). After I had infected her with a *rhesus*-strain (*Jc.*), and recovered it from her in culture (see Table X, p. 35, and Scheme C, p. 36), I re-treated her with emetine and once more eradicated her *E. histolytica* completely. This treatment has not been described previously, but was almost identical with that already recorded[1]. I gave Susanna 60 mg. of emetine bismuth iodide daily from March 10 to March 15, 1929 (0·36 g. in 6 days), and examined her faeces every day during and after treatment—both culturally and microscopically. On the third day of treatment (12. iii. 29) the examinations were both negative, and remained so thereafter.

On 16 April 1929, when the monkey was completely recovered, and her faeces had shown no *E. histolytica* infection for 36 consecutive days (daily microscopic and cultural examinations—except for 2 days after treatment, when no faeces at all were passed), I fed her on culture-cysts of Strain *NRK*. I gave her all the cysts, which were very numerous, in two cultures (33rd serial subculture, made on 11. iv. 29, taken from the incubator on 13. iv. 29, and kept at room-temperature for 3 days before feeding).

Susanna promptly acquired infection again, *E. histolytica* being found microscopically and culturally on the fourth day after the experimental feeding: and she remained infected, with a strain indistinguishable from the original Strain *NRK*., until I again eradicated it in October, 1929 (6 months later). The experiment is summarized in Table XII.

Table XII.

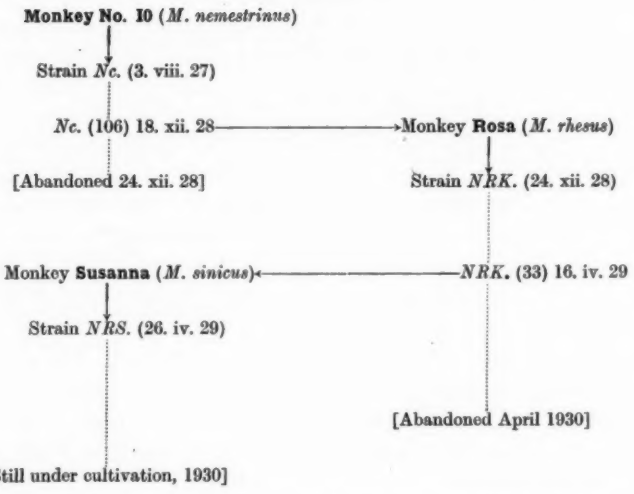
Susanna (*M. sinicus*) fed with culture-cysts (*NRK*.) from *M. rhesus* (Rosa) infected from *M. nemestrinus*. 16. iv. 29		Dates of Examinations (1929)						
		Mar. 12–Apr. 16	17	18	19	20	21	22
E. histolytica	faeces	–	–	–	–	+	+	+
	cultures	–	–	–	–	+	+	+

Whilst she was parasitized by this strain of *E. histolytica*, Susanna never displayed any symptoms of dysentery or showed any signs of intestinal or general discomfort. She remained perfectly healthy throughout—like all my other monkeys infected, either naturally or experimentally, with *E. histolytica macacorum*. I isolated and studied several strains of amoebae from her faeces, and have kept one of them (*NRS*.) continuously *in vitro* since 26 April 1929. All these strains were morphologically and culturally identical with Strains *Nc.* and *NRK*., from which they were descended.

The whole experiment just described—the transmission of a pure strain of *E. histolytica* from *M. nemestrinus* to *M. rhesus* and thence to *M. sinicus*—is summarized in Scheme D.

[1] Dobell with Bishop (1929), p. 457; *ibid.* Table VIII, p. 467.

Scheme D.


Monkey No. 10 (M. nemestrinus)
Strain Nc. (3. viii. 27)
Nc. (106) 18. xii. 28
Monkey Rosa (M. rhesus)
[Abandoned 24. xii. 28]
Strain NRK. (24. xii. 28)
Monkey Susanna (M. sinicus)
NRK. (33) 16. iv. 29
Strain NRS. (26. iv. 29)
[Abandoned April 1930]
[Still under cultivation, 1930]

The three series of experiments just related, notwithstanding their obvious inadequacy from a statistical standpoint, have convinced me that the strains of *E. histolytica* occurring naturally in *M. sinicus*, *M. rhesus*, and *M. nemestrinus* are readily interchangeable among these three hosts. All my attempts at cross-infection were so uniformly successful and so carefully controlled in every way, that I am fully satisfied with their main results. It is clear that some simian strains, at least, are not only morphologically and culturally indistinguishable, but any one of them is also readily transmissible from one species of *Macacus* to another. It is as easy to infect a *sinicus* with a strain derived from *rhesus* or *nemestrinus* as with a strain from another *sinicus*.

A few other points elucidated by these experiments may also be noted here. In the first place, the experiments showed that prolonged cultivation of *E. histolytica macacorum* does not deprive it of infectivity for macaques. The strains which I used were propagated in various artificial media—with rice-starch and without—and remained equally infective for all periods up to about a year and a half. This is in striking contrast with the loss of infectivity for kittens demonstrated in strains of the same parasite cultivated in similar media for like periods.

Moreover, the experiments as a whole have convinced me that morphologically normal cysts of *E. histolytica* formed in cultures are capable of fulfilling their normal physiological rôle. When administered by the natural route *per os* they can infect a monkey quite as effectively as cysts formed naturally in the intestine. All my monkeys fed with culture-cysts not only acquired infection promptly, but they also remained infected until they were killed or their infections were eradicated by treatment.

The rapidity with which the infections were established is shown by the shortness of the latent ("incubation") period following the experimental feeding. In the four experiments made, the latent period was only 2 to 4 days (see Table XIII, which collects data already recorded in Tables IX to XII).

Table XIII.

Monkey	Species	Strain of amoebae inoculated	Latent period
Polo	*M. sinicus*	*K.28c.* [*sinicus*]	3 days
Susanna	*M. sinicus*	*Jc.* [*rhesus*]	2 days
Rosa	*M. rhesus*	*Nc.* [*nemestrinus*]	3 days
Susanna	*M. sinicus*	*NRK.* [*nemestrinus* from *rhesus*]	4 days

It must be remembered, however, that *E. histolytica* was usually first found in the faeces of experimentally-fed animals by cultural methods. (As a rule, the amoebae were recovered in cultures a day earlier than they were discoverable by direct microscopic examination.) And it must also be remembered that massive doses of cysts were administered to each monkey. Each swallowed, in the experiment, many thousands of ripe, normal, and fresh (1–3 days old) culture-cysts—a dose far in excess of anything it would be likely to ingest under natural conditions.

Summary of the Foregoing Experiments (pp. 31–40).

Attempts to transmit strains of *E. histolytica macacorum* from one species of *Macacus* to another have been uniformly successful.

All the strains used were isolated in pure culture, and carefully tested and studied before trial: and all the macaques employed were previously freed from infection with *E. histolytica* by treatment with emetine, and proved to be "clean" by prolonged daily examination of their faeces—both microscopically and culturally.

With such material and precautions, it has been found possible to transmit a strain of *E. histolytica* from one *Macacus sinicus* to another *M. sinicus*: a strain from *M. rhesus* to *M. sinicus*: and a strain from *M. nemestrinus* to *M. rhesus* and thence to *M. sinicus*.

These experiments have shown that strains of *E. histolytica macacorum* may be cultivated in various media for any period up to about a year and a half without losing their infectivity for macaques. They have also shown that normal culture-cysts are just as infective as cysts formed naturally in the intestine.

The strains of *E. histolytica* employed were all derived from healthy non-dysenteric macaques, and produced no dysentery on introduction into their new hosts.

All macaques became demonstrably infected within 4 days at most after ingestion of culture-cysts of *E. histolytica macacorum*: and in every case the induced infection persisted for the duration of the experiment (maximum period 15½ months).

3. EXPERIMENTAL INFECTION OF MACAQUES WITH *E. HISTOLYTICA HOMINIS*.

For this series of experiments I used the same methods as in the previous series: but instead of using various strains of amoebae isolated from macaques, I used throughout a single pure strain (*D.*)—or its derivatives—obtained from a human being. My main object was to discover whether *M. sinicus* and *M. rhesus*, when cured of their natural infections with *E. histolytica macacorum*, can be reinfected with *E. histolytica hominis*.

Strain D.—The strain of *E. histolytica hominis* employed was originally cultivated, from a patient suffering from acute amoebic dysentery, by Dr J. Drbohlav[1]. It was isolated by him in London on 3 November 1924, and he kindly gave me a culture (11th serial subculture) on 24 November 1924. I have maintained this strain *in vitro* ever since, and it has been very thoroughly studied and used for numerous experiments[2]. For the greater part of its history it has been cultivated in horse-serum and Ringer-eggwhite medium with rice-starch (HSre + S, Dobell-Laidlaw), in which it grows luxuriantly but does not now form cysts. During the last 5 years it has usually been transplanted once a week—the cultures always living for at least 7 and often for 10 days.

(i) *Transmission of Strain* D. *to* M. sinicus.

I first attempted to transmit Strain *D.* to a clean *M. sinicus* in April 1927, when it had been under continuous cultivation *in vitro* for approximately $2\frac{1}{2}$ years.

The monkey used for this experiment was Mungo, whose natural infections with *E. histolytica* had been removed by treatment with emetine[3]. He was fed with all the cysts in three cultures (serial subculture 303)—in which they were not very numerous, though normal and fresh—on 19 April 1927, and soon became infected. That he was uninfected before the experiment was made is evidenced by the fact that his faeces had been very carefully examined, both microscopically and culturally, every day for the preceding 43 days (6 weeks, including 3 days during treatment) with consistently negative results[4]. The experiment is summarized in Table XIV (p. 42).

Mungo became heavily infected, and the infection persisted for the rest of his life (nearly 16 months). He continued to pass amoebae or cysts of *E. histolytica*, morphologically and culturally indistinguishable from those of Strain *D.*, until I killed him on 8 August 1928. During this time he never suffered from dysentery, but remained normal and healthy—growing rapidly, and in full vigour throughout. At necropsy I could discover no trace of amoebic ulceration in his intestines.

[1] See Drbohlav (1925, 1925*a*); Dobell and Laidlaw (1926, 1926*a*).

[2] See Dobell and Laidlaw (1926, 1926*a*); Laidlaw, Dobell, and Bishop (1928); Dobell (1928). Dr Laidlaw and Miss Bishop have kindly assisted in the propagation of this strain from time to time, but it has been in my hands during most of the last $5\frac{1}{2}$ years.

[3] See Dobell with Bishop (1929), p. 455; *ibid.* Table VI, p. 467.

[4] An unsuccessful experiment alluded to on p. 12 is included in this negative period.

Table XIV.

Mungo (*M. sinicus*) fed with culture-cysts of *E. h. hominis* (Strain *D.*), serial subculture 303. 19. iv. 27		Dates of Examinations (1927)							
		Mar. 7–Apr. 19	20	21	22	23	24	25	26
E. histolytica	faeces	–	–	–	–	–	+	+	+
	cultures	–	–	–	–	+	+	+	+

I studied various strains of *E. histolytica* isolated from this monkey after his experimental infection, but especially one, called *DM.*, which was used for the next experiment.

Strain DM. was isolated from cysts in Mungo's faeces on 28 April 1927, and I have cultivated it continuously ever since. It has been grown throughout in egg and horse-serum medium with rice-starch (Ehs + S, Dobell-Laidlaw), in which it develops abundantly. The cultures live regularly for 8 to 10 days, but transplantation has generally been made once a week. In this medium, with its original bacterial flora, no cysts have ever been formed by this strain during the $3\frac{1}{4}$ years it has been under cultivation: but rich crops of cysts can be obtained from it at any desired moment by the methods already described[1].

After keeping and studying this strain (*E. h. hominis* from *M. sinicus*) for 2 years I attempted to transmit it, by means of cysts, to a macaque of different species (*M. rhesus*). This experiment must now be described.

(ii) *Transmission of Strain* D. *from* sinicus *to* rhesus.

The monkey used for this attempt was Rosa (*M. rhesus*), whose own natural infection with *E. histolytica* was removed by emetine-treatment as elsewhere recorded[2], and who had afterwards been experimentally reinfected with another strain derived from *M. nemestrinus*—as described on an earlier page[3]. This infection was afterwards eradicated by the same method. From 10. iii. 29 to 15. iii. 29 I gave Rosa 60 mg. of emetine bismuth iodide daily (0·36 g. in 6 consecutive days), and examined her faeces every day—both microscopically and culturally—before, during, and after treatment. *E. histolytica* disappeared completely on the third day of treatment, and Rosa's faeces then remained negative for the next 5 weeks (34 consecutive daily examinations). As I felt satisfied that her infection had been removed, and as she was then quite recovered and normal in every way, I performed the present experiment with her.

On 16 April 1929 I fed Rosa with all the cysts in two cultures (121st serial subculture) prepared from Strain *DM.* These cysts were 3 days old, and very numerous (many thousands, at least); and they were normal, ripe, and alive—as proved by careful control experiments[4].

[1] See p. 5 *supra.*

[2] Dobell with Bishop (1929), p. 458; *ibid.* Table IX, p. 467.

[3] See p. 37 *supra.*

[4] Well fixed and stained preparations—made from a small sample of the material fed—showed abundant normal 4-nucleate cysts: and another small sample, inoculated into a culture-tube and incubated, hatched normally and produced an excellent culture.

I continued to examine Rosa's faeces every day, but to my surprise they remained consistently negative for the next 18 days. As this was far beyond the latent period observed in any of my earlier experiments, I began to think that the attempt had failed. It seemed possible that Strain *DM*. had lost its infectivity through prolonged cultivation. In order to make certain whether this were so or not, I therefore administered massive doses of similar cysts every day for 5 days running (May 5–9, inclusive). All the cysts—carefully controlled—in a single rich culture were administered each day.

After this intensive treatment Rosa became infected, but the infection developed erratically—as judged by the daily microscopic and cultural examinations—and did not become firmly established until more than a week (about 9 days) after the last dose of cysts was given. The whole experiment is summarized in Table XV: but it should be added that most of the early

Table XV.

Rosa (*M. rhesus*) fed with culture-cysts of Strain *DM*. from Mungo (*M. sinicus*) infected with *E. h. hominis* (Strain *D.*). 16. iv. 29

	Dates of Examinations (1929)																
	Mar. 14–Apr. 16	Apr. 17–May 4	5*	6*	7*	8*	9*	10	11	12	13	14	15	16	17	18
E. histolytica faeces	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	+
E. histolytica cultures	–	–	–	–	–	+	+	+	–	–	–	+	+	+	+	+

* Dates on which cysts were again fed.

positive findings (May 8–16) were obtained culturally with great difficulty. Both faeces and primary cultures were frequently negative, and a positive result was only reached by further cultivation of such originally "negative" material. Moreover, it is possible that the positive cultures ultimately obtained from faeces passed on May 8, 9, and 10, were really derived from cysts—administered to the monkey on previous days—which had passed through the intestine without hatching[1]. They cannot be taken to prove that infection was established on any of these dates. Amoebae were not microscopically discoverable in the intestinal evacuations until May 15 (6 days after the last feeding on cysts).

Though the interpretation of details in this experiment is obviously debatable[2], its main result is certain. Rosa undoubtedly acquired an infection with Strain *DM*., and retained it for the duration of the subsequent period of observation. After 18 May 1929 *E. histolytica* was almost invariably discoverable in her faeces by direct microscopic examination, or recoverable from them by cultivation. The infection persisted for nearly 5 months (until 6. x. 29), when I removed it by treatment with emetine. During this period the monkey

[1] I have elsewhere (1927) adduced evidence to show that cysts of *E. histolytica* may sometimes live for as long as 24 hours at 37° C. without hatching, though still capable of hatching a little later: and there can be no doubt that food frequently passes through the intestine of *M. rhesus* in much less than 24 hours.

[2] Some further light is thrown on this subject by a later experiment: see p. 46 *infra*.

herself remained normal. She never suffered from dysentery or any other complaint, and continued to grow at her normal rate. During this period, also, I isolated and studied several strains of *E. histolytica* from her faeces. They were all typical in every way, and morphologically and culturally indistinguishable from Strains *D.* and *DM.*, from which they were derived. One such strain (*DMR.*) was studied particularly; and as it will be mentioned again, its origin must be noted here.

Strain DMR.—This strain was isolated from Rosa's faeces on 15 May 1929, and has been cultivated continuously ever since. It has been grown throughout, in the amoeboid state, in Ehs medium (Dobell-Laidlaw) without starch; but it encysts regularly and abundantly whenever starch is supplied to it, and has continued to develop for more than a year exactly like Strain *K*.28c.[1] Morphologically it is not to be distinguished from Strains *D.* and *DM.*, from which it is descended, but one of its physiological peculiarities will be alluded to in another connexion later[2].

(iii) *Transmission of Strain* D. *to* sinicus *and* rhesus *after Prolonged Cultivation* (5 *years*).

The experiment just described seemed to indicate that *E. histolytica hominis* might lose its infectivity for macaques as a result of prolonged cultivation[3]. I had previously found that continued maintenance *in vitro* caused several strains of the parasite (both *macacorum* and *hominis*) to lose their power of infecting kittens; though all strains of *E. histolytica macacorum* remained fully infective for macaques for as long as they were cultivated (up to about a year and a half), and Strain *D.* itself (*hominis*) had remained infective for *M. sinicus* for 2½ years. However, the difficulty I met with in transmitting Strain *DM.* to Rosa suggested that it was becoming non-infective for macaques as well as for kittens[4].

I therefore made a final experiment with two monkeys (a *sinicus* and a *rhesus*), in order to find out whether Strain *D.* had really lost its infectivity for macaques after prolonged propagation *in vitro*. The experiment was made in November 1929, when the strain had been cultivated continuously in the amoeboid state, without passage through any animal, for 5 years.

The two monkeys used for this trial were Susanna (*M. sinicus*) and Rosa (*M. rhesus*). Susanna was cured of her natural infection—as already described—by treatment with emetine[5]. She was then reinfected with a strain (*Jc.*) of *E. histolytica* derived from *M. rhesus*[6]: again cured of this infection by similar

[1] See Dobell (1928). [2] See p. 58 *infra*.

[3] It will be remembered that Strain *DM.*, with which the experiment was made, had been under continuous cultivation for 2 years. It was derived, moreover, from a monkey (Mungo) in which it had sojourned for only 9 days, after earlier maintenance *in vitro* (as "Strain *D.*") for some 2½ years. Apart from its short period of passage through Mungo, therefore, Strain *D.* + *DM.* had been kept *in vitro* for almost 4½ years.

[4] Strain *DM.* was tested only once on a kitten (with negative result). The experiment is described later (p. 52).

[5] Dobell with Bishop (1929), p. 457. [6] See p. 35 *supra*.

treatment[1]: and again reinfected with a strain (*NRK.*) derived from *M. nemestrinus*, after passage through *M. rhesus*[2]. This infection I also removed, by the same methods, for the purpose of the present experiment. From October 6 to 10 inclusive, 1929, I gave Susanna 60 mg. of emetine bismuth iodide daily (0·3 g. in all) for 5 consecutive days[3], and examined her faeces every day—both culturally and microscopically—during and after treatment. They became completely negative for *E. histolytica* after treatment, and remained so for as long as I continued the examinations (11. x. 29—26. xi. 29 = nearly 7 weeks).

Rosa (*M. rhesus*), the other monkey used, had had her original infection with *E. histolytica* eradicated by similar treatment[4]. She was afterwards reinfected with a strain (*Nc.*) derived from *M. nemestrinus*[5], and again cured with emetine[6]. She was then reinfected with a human strain (*DM.*) after passage through *M. sinicus*[7]. This infection I once more cured by similar means. I gave Rosa 60 mg. of emetine bismuth iodide daily for 6 consecutive days (6–11 October 1929), and made careful daily examinations of her faeces—both microscopically and culturally—during and after treatment. They were all completely negative for 7 weeks, so I concluded that she had been cured and used her for the present experiment.

Susanna was fed with culture-cysts of Strain *D.* (serial subculture No. 462) on 26 November 1929. The daily examinations of her faeces were continued, and *E. histolytica* appeared in the cultures 4 days later (30. xi. 29): but no amoebae or cysts were discoverable by direct microscopic examination until 6 days after the experimental feeding (first found on 2. xii. 29). The infection became firmly established, and has persisted ever since. Since the beginning of December 1929, I have had no difficulty in discovering *E. histolytica* in Susanna's faeces, and in recovering this species in cultures made therefrom, whenever I have attempted to do so.

This experiment is summarized in Table XVI, which shows that Strain *D.* retained its infectivity undiminished for *M. sinicus* for 5 years. (The Table may be compared with Table XIV, p. 42; which summarizes a similar experiment made with the same strain 2½ years earlier.)

Table XVI.

Susanna (*M. sinicus*) fed with culture-cysts of Strain *D.* (*E. h. hominis*) serial subculture 462. 26. xi. 29		Dates of Examinations (1929)							
							Dec.		
		Oct. 11–Nov. 26	27	28	29	30	1	2	3
E. histolytica	faeces	–	–	–	–	–	–	+	+
	cultures	–	–	–	–	+	+	+	+

[1] See p. 38 *supra*. [2] See p. 38 *supra*.

[3] On this occasion—her third treatment with emetine—I was unable to continue the administration of the alkaloid for a longer period, because it made the monkey so ill. But though obviously poisoned she recovered rapidly afterwards, and has remained normal ever since.

[4] See Dobell with Bishop (1929), p. 458.

[5] See p. 37 *supra*. [6] See p. 42 *supra*. [7] See p. 43 *supra*.

Rosa (*M. rhesus*), after her third cure with emetine, was fed on numerous excellent cysts of Strain *D.* (serial subculture No. 461) on 25 November 1929, after her stools had been consistently negative for *E. histolytica* during 7 weeks. The daily examinations were continued; but to my disappointment—because of the successful result obtained in the concurrent experiment with Susanna (Table XVI)—they remained completely negative for the next 18 days. I therefore feared that the attempted infection had failed. But as the cysts administered to Rosa had been very carefully tested, and proved to be normal and viable, I was loath to regard the experiment as a failure, or to resort to more drastic dosage with similar cysts—as in my earlier attempts to infect this monkey with Strain *DM.* (cf. Table XV, p. 43). It seemed just possible that Strain *D.* (or its derivatives, such as *DM.*) might develop abnormally slowly, with an unusually prolonged latent period, in the particular monkey (Rosa) used for the experiment. I therefore resolved to continue my daily examinations further—for months if necessary—in order to exclude this possibility, or to prove that Strain *D.* (though still infective for *M. sinicus*) had become non-infective for *M. rhesus*.

But I was not called upon to make such protracted negative observations. On the 19th day after the administration of the infective cysts, the culture made from Rosa's faeces showed a few active forms of *E. histolytica*, though the microscopic examination was negative: while on the 20th day I not only found amoebae in the culture, but also discovered numerous typical cysts in the sample of faeces from which the culture was made. After this date (15. xii. 29) *E. histolytica* appeared regularly in the stools, and the infection has persisted ever since. For the last 6 months Rosa—like Susanna—has remained infected with a strain of the parasite which is always readily demonstrable by microscopic or cultural examination, and which is indistinguishable morphologically from Strain *D.*

The experiment is summarized in the accompanying Table XVII.

Table XVII.

Rosa (*M. rhesus*) fed with culture-cysts of Strain *D.* (*E. h. hominis*) serial subculture 461. 25. xi. 29		Dates of Examinations (1929)					
		Oct. 10–Nov. 25	Nov. 26–Dec. 13	14	15	16	17
E. histolytica	faeces	−	−	−	+	+	+
	cultures	−	−	+	+	+	+

This experiment threw light upon my previous results with Strain *DM.* (Table XV, p. 43): for it showed that Strain *D.* developed abnormally slowly in Rosa—the latent period ("incubation period") being four or five times as long as in any other experiment. It also showed that in my experiment with Strain *DM.* I ought to have waited for more than 18 days before I concluded that the first attempt at infection was a failure. It is not now certain whether it was or not. All that can be concluded from that experiment is that it succeeded in its main object. I transmitted Strain *DM.* to Rosa by feeding her

on cysts, but I fed her too often for it to be possible to determine either the exact date of infection or the latent period.

My two experiments with Strains *D.* and *DM.*, using Rosa (*M. rhesus*) as the subject, indicate that this monkey is less readily infectible with *D.* and its derivatives than the two other monkeys tested. These two (Mungo and Susanna) were of a different species (*M. sinicus*); but there are, at present, no reasons for supposing that *M. rhesus* is less easily infected than *M. sinicus* with all human strains of *E. histolytica*. Considering my other results, I can only ascribe the difference in behaviour to some unknown individual peculiarity of Rosa herself.

It is clear, from the last experiments made with Susanna and Rosa (Tables XVI and XVII), that Strain *D.* has not lost its infectivity for either *M. sinicus* or *M. rhesus* even after continuous cultivation for a prolonged period. As far as I can judge, from my limited experience, its infectivity for macaques has undergone no change in the last 5 years. I conclude, therefore, that a strain of *E. histolytica hominis* may be transmitted to *M. sinicus* and *M. rhesus* quite as readily as a strain of *E. histolytica macacorum*: and that, when propagated *in vitro*, it can retain its infectivity for macaques for an indefinite time—certainly for 5 years at least.

Since I infected Susanna and Rosa with *E. histolytica hominis* (Strain *D.*) in November, 1929, I have kept these two monkeys together, but completely isolated from all other monkeys and every source of contagion from human beings. Neither has developed any dysenteric symptoms, but both have remained perfectly healthy though heavily infected with a strain of *E. histolytica* originally pathogenic in Man. At various times I have isolated and studied strains of the parasite from their faeces: all have been closely similar, and morphologically indistinguishable from the original Strain *D.* with which they were experimentally infected. Two of these strains (*DS.* from Susanna and *DRK.* from Rosa) have been particularly studied, and will be referred to again later[1].

In order to make clear my various experiments with Strain *D.* and its derivatives (described in the present section), I have summarized them graphically in the accompanying Scheme E (p. 48): but as the reader may find difficulty in following the various experiments performed with the two monkeys Susanna and Rosa—which were used for other experiments with *E. histolytica macacorum* (described in an earlier section)—I have briefly recapitulated the infections of these animals in Tables XVIII and XIX (p. 48).

[1] See pp. 53, 58 *infra*.

Scheme E.

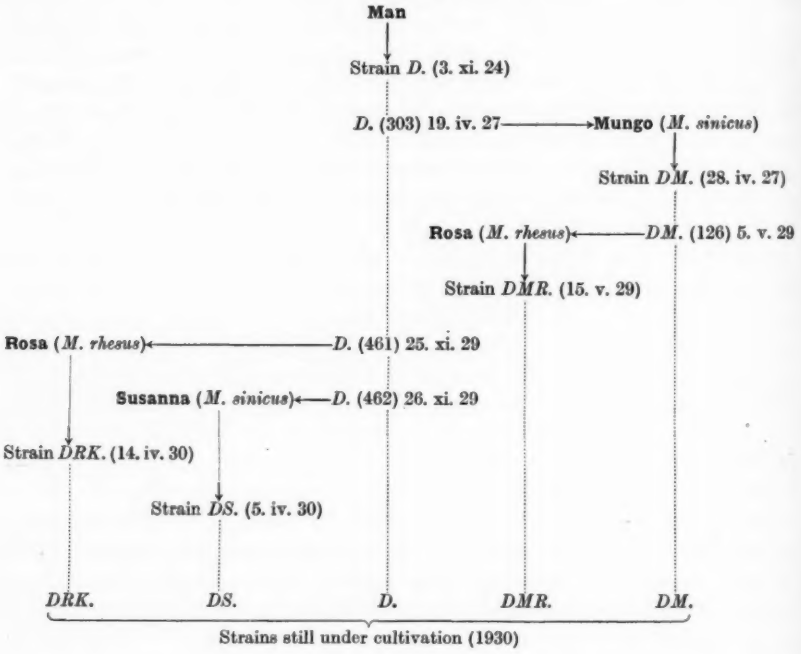


Table XVIII.

Susanna (*M. sinicus*)

Original infection	Experimental reinfections		
	1	2	3
E. histolytica macacorum (24. vii. 24–7. x. 28). Removed by treatment with emetine 7. x. 28–12. x. 28.	Strain *Jc.* from *M. rhesus*. 18. xii. 28. Removed with emetine 10. iii. 29–15. iii. 29	Strain *NRK.* from *M. rhesus* infected from *M. nemestrinus*. 16. iv. 29. Removed with emetine 6. x. 29–10. x. 29	Strain *D.* (*hominis*) 26. xi. 29. Monkey still infected (July 1930)

Table XIX.

Rosa (*M. rhesus*)

Original infection	Experimental reinfections		
	1	2	3
E. histolytica macacorum (... x. 26–7. x. 28). Removed by treatment with emetine 7. x. 28–13. x. 28	Strain *Nc.* from *M. nemestrinus*. 18. xii. 28. Removed with emetine 10. iii. 29–15. iii. 29	Strain *DM.* from *M. sinicus* infected from Man. 16. iv. 29. Removed with emetine 6. x. 29–11. x. 29	Strain *D.* (*hominis*) 25. xi. 29. Monkey still infected (July 1930)

SUMMARY OF THE FOREGOING EXPERIMENTS (pp. 41–48).

A strain (*D.*) of *E. histolytica*, derived from a man suffering from acute amoebic dysentery, has been cultivated *in vitro* continuously for 5½ years.

After cultivation for 2½ years it was successfully transmitted to *M. sinicus*. On recovery from the experimentally infected monkey, its derivative (*DM.*) was further cultivated for 2 years, and then transmitted to *M. rhesus*.

The original strain itself (*D.*) has also been directly transmitted to both *M. sinicus* and *M. rhesus* after continuous cultivation for 5 years.

It may therefore be concluded that *E. histolytica hominis* is transmissible to *M. sinicus* and *M. rhesus*, even after prolonged cultivation *in vitro*; and that the infection induced in one species of macaque is communicable to the other.

In all the monkeys (only three in number) infected experimentally, no dysenteric symptoms or lesions were observable at any time. *E. histolytica hominis*, when artificially implanted into my macaques, has hitherto produced persistent[1] infections in no way distinguishable from their own original and harmless infections with *E. histolytica macacorum*.

4. SUPPLEMENTARY EXPERIMENTS.

In addition to the experiments described in the three preceding subsections (1–3), I have made various others with the object of elucidating sundry minor details. These accessory researches were of a somewhat miscellaneous character, but they fall roughly into two groups: (i) tests made to determine the infectivity, for kittens, of various strains or substrains of *E. histolytica* whose origins have, for the most part, been described already; and (ii) the investigation of another physiological property of these strains—namely, their ability to ingest human red blood-corpuscles *in vitro*.

(i) *The Infectivity of various Strains of* E. histolytica *for Kittens.*

It has already been mentioned that several strains of *E. histolytica*, studied in the course of the present researches, lost their power of infecting kittens—apparently as a result of protracted cultivation outside the body. This observation was originally made in the case of Strain *D.*, whose behaviour may therefore be recapitulated here first.

Strain D.—This strain (*E. h. hominis*, derived from a man afflicted with acute amoebic dysentery[2]) when freshly isolated was found (by Dr Drbohlav) to be capable of transmission to kittens; but after it had been maintained *in vitro* (by Dr Laidlaw and myself) for about 2 months, it lost its infectivity completely. It was isolated at the beginning of November 1924, and in the first two months of 1925 I made 8 very carefully controlled attempts to infect 7 different kittens with it: but they were all completely unsuccessful[3]. Since

[1] *I.e.* the infections have persisted for the duration of the period of observation (maximum time 16 months).

[2] Cf. p. 41 *supra*.

[3] See Dobell and Laidlaw (1926*a*), pp. 314 *et seq.*

then I have made two further attempts, after cultivation of the strain for $2\frac{1}{2}$ and $5\frac{1}{2}$ years respectively, with the same result.

The later experiments were made with two healthy young kittens (both just 6 weeks old), and with every possible precaution. Very rich cultures of amoebae were inoculated into these animals (Nos. 41 and 42), and both were studied in great detail afterwards. I examined almost every stool passed by either kitten both microscopically and culturally. One (No. 41) I killed after 20 days: the other (No. 42) is still alive and well. Neither contracted dysentery, and all microscopic, cultural, and *post-mortem* findings were completely negative.

From all these carefully conducted experiments I am personally satisfied that Strain *D.* was—at the times of trial—incapable of infecting any of the kittens used: and it seems to me justifiable to conclude, therefore, that this strain has lost its infectivity for kittens generally.

All my attempts to transmit Strain *D.* to kittens are summarized in Table XX, which includes the results of eight experiments recorded elsewhere[1].

Table XX. *Attempts to transmit Strain* D. (E. h. hominis, *isolated* 3. xi. 24) *to Kittens.*

Kitten No.	Date of inoculation	Serial sub-culture No.	Age of strain since isolation	Result
4	8. i. 25	26	66 days	Negative
5	8. i. 25	26	66 „	„
6	8. i. 25	26	66 „	„
10	23. i. 25	33	81 „	„
11	8. i. 25	26	66 „	„
	29. i. 25	36	87 „	„
12	29. i. 25	36	87 „	„
13	13. ii. 25	46	102 „	„
41	4. v. 27	311	$2\frac{1}{2}$ years	All microscopic and cultural examinations negative till 24. v. 27, when killed. No amoebae or amoebic lesions found *post mortem*
42	2. iv. 30	471	$5\frac{1}{2}$ „	All microscopic and cultural examinations negative till 1. v. 30, when discharged as normal. [Still alive and well, July 1930]

For further elucidation of certain discrepancies between the behaviour of Strain *D.* (*E. h. hominis*) and various strains of *E. h. macacorum*, I needed another human strain from a non-dysenteric subject. This strain (*F.*) I obtained by chance from a sample of faeces—from a patient whose history I subsequently learnt—which was sent to me for diagnosis: its origin and history may be briefly noted here[2].

Strain F. was isolated on 25 August 1925 from the faeces of a patient who was not suffering, and who had never previously suffered, from dysentery.

[1] By Dobell and Laidlaw (1926*a*), Table IV, p. 316, where further details are given of the experiments here entered merely as "negative."

[2] Previously mentioned by Dobell and Laidlaw (1926*a*), p. 285.

His malady at the time was, however, diagnosed clinically as due to "intestinal sepsis" (of unknown aetiology[1]).

I inoculated the second serial culture of Strain *F.* intrarectally into a normal kitten (No. 24, then about 8 weeks old) on 28 August 1925. Kitten 24 promptly became infected—passing blood, mucus, and amoebae with its faeces 3 days later. It suffered from typical acute amoebic dysentery until 8 September 1925 (9 days); when I killed it, as a fatal issue appeared imminent. I isolated and studied half-a-dozen substrains of Strain *F.* from this kitten's dejecta on different days, and propagated one of them—isolated on 1. ix. 25, and known as *K.*[2]24—for over 5 months *in vitro*, when I relinquished it voluntarily at the 34th serial subculture (10. ii. 26).

At necropsy (8. ix. 25) Kitten 24 showed typical amoebic lesions in its large intestine, with no secondary involvement of any other organ.

The original strain (*F.*) I cultivated until 18. ix. 25 (14 serial subcultures in 24 days) and then abandoned, as it had supplied me with all the information which I required. It behaved[2], as regards its infectivity for a kitten, as a typical *E. histolytica hominis*—notwithstanding that it originated from a non-dysenteric human being. Some other properties of Strain *F.* and its feline derivatives will be noted in the next subsection[3].

Strain P.—It may be recalled that Strain *K.*28c., which has served for so many experiments, was originally derived from Bonar (*M. sinicus*), and after passage through a kitten (No. 28) was transmitted back to another *M. sinicus* (Polo)[4]. On cultivation Strain *K.*28c. itself rapidly lost its infectivity for kittens[5], but it became necessary to ascertain whether the infectivity had been restored by reimplantation in a monkey of the same species as its original host. Although I studied many strains of *E. histolytica* from Polo after his experimental infection with *K.*28c., I was able to make only one attempt to transmit them to kittens.

For this attempt I used a primary culture made from Polo's faeces on 2 May 1927 (23 days after infection with Strain *K.*28c.). I purposely used a primary culture—though I was naturally unable to test the purity of the amoebae at so early a stage in the history of the strain—lest continued cultivation might impair the infectivity. By this date, however, I had satisfied myself that Polo's faeces contained only one pure strain of *E. histolytica*—that which I had implanted in him experimentally. But to make assurance doubly sure, I continued the strain cultivated on May 2 for 15 days (8 cultural generations—

[1] But probably—as subsequent events showed—primarily caused by infection with *E. histolytica*. This patient was treated with emetine, and cured of his infection: whereupon his clinical condition improved rapidly, and he made a complete recovery. Through the courtesy of Dr H. C. Sinderson and Sir William Willcox I was able to examine the patient's stools four times during the third week after treatment with emetine, and again on three occasions two years later (November, 1927). All these examinations—made both microscopically and culturally—were completely negative.

[2] The action of emetine on this strain *in vitro* has been described elsewhere (Dobell and Laidlaw, 1926).

[3] See p. 57 *infra*.

[4] See Scheme B, p. 34.

[5] See Table VII, p. 27.

28 cultures altogether); I obtained typical cysts from it in 4 cultures; and I hatched 3 lots of these in order to make certain that they were normal. I am confident, therefore, that the strain (*P.*) used in this experiment was pure and authentic.

The kitten used (No. 40), a normal healthy little animal under my eye from birth, was 43 days old at the date of the experiment. It was intrarectally inoculated (with a very rich culture of amoebae) on 3 May 1927, and afterwards passed no faeces for 2 days. Thereafter it remained healthy until I killed it 20 days later. It never contracted dysentery, and its faeces remained normal. They were examined repeatedly with the microscope; and on 8 different days I attempted to isolate *E. histolytica* from them in cultures, but always with negative results. At necropsy, also, the kitten appeared perfectly normal. No amoebic lesions were discoverable in its large intestine, and no amoebae could be found microscopically or culturally in its intestinal contents.

It is thus certain that Strain *P.* did not infect the only kitten on which it was tried. By itself, however, this experiment is inconclusive—for several obvious reasons. For example, Strain *K.*28c. originally displayed only feeble pathogenicity for kittens, and such infectivity as it possessed was soon lost on cultivation: while one single negative experiment with its derivative, Strain *P.*, is manifestly insufficient to prove that all strains from Polo were non-infective for all kittens. Nevertheless, I was able to make some further confirmatory observations with other similar strains, though all of them were derived originally from Strain *D.* (*E. h. hominis*).

Strain DM.—As already noted, Strain *D.*, after it had lost its infectivity for kittens[1], was experimentally transmitted to Mungo (*M. sinicus*)[2], in whom it became firmly established. From this monkey's faeces I isolated several pure strains, and with one of these (*DM.*, still under cultivation[3]) I attempted to infect a kitten.

For this experiment I used the primary culture[4] (extremely rich) of Strain *DM.* made from Mungo's faeces on 29 April 1927 (10 days after his experimental infection with Strain *D.*). After incubation for 3 days it was inoculated (2. v. 27) intrarectally into Kitten 39—a normal healthy animal just 6 weeks old. No infection followed. After inoculation, the kitten passed no faeces for 5 days: and it then defaecated at infrequent intervals till I killed it 3 weeks later (23. v. 27). During this period I examined a sample of every stool deposited (only 7) both microscopically and culturally, but with completely negative results. No amoebic lesions were visible at necropsy, and in a culture made from the intestinal contents *post mortem* no *E. histolytica* could be found.

[1] See Table XX, p. 50 *supra*; and Dobell and Laidlaw (1926*a*), Table IV, p. 316.

[2] See Table XIV, p. 42 *supra*.

[3] See p. 42 and Scheme E, p. 48 *supra*.

[4] For reasons similar to those already given in regard to Strain *P.* (p. 51), and with similar safeguards.

It appears certain, therefore, that Strain *DM.* was non-infective to Kitten 39.

This experiment again, however, was not—by itself—conclusive: so in order to make sure that a strain of *E. histolytica* which had lost its infectivity for kittens by prolonged cultivation *in vitro* could not regain this power by passage through a macaque, I made four further experiments with other derivatives of Strain *D.*

At the time when these trials were made (April–May, 1930), Strain *D.* itself had undoubtedly lost its infectivity for kittens[1]. But when it had been kept *in vitro* for 5 years I implanted it, as already described[2], into Susanna (*M. sinicus*) and Rosa (*M. rhesus*); and I allowed these monkeys to remain infected for 5 months[3] before I attempted to transmit their infections. I then isolated 2 pure strains from each macaque, and inoculated them with every precaution into 4 healthy kittens.

The 4 strains of *E. histolytica* used in these experiments were:

Strain DR.—Isolated 14 April 1930 from the faeces of Rosa (*M. rhesus*), experimentally infected with Strain *D.* on 25 November 1929. Grown throughout, with the bacterial flora which naturally accompanied it, in HSre + S medium (Dobell-Laidlaw); voluntarily abandoned 29. iv. 30.

Strain DRK.—Isolated from the same material as the preceding, but grown —after the first few cultural generations—in Ehs medium (Dobell-Laidlaw; a slight modification of the original medium of Boeck and Drbohlav) without starch, but with addition of the bacterial flora accompanying Strain *K.*28c. Still under cultivation.

Strain DS.—Isolated 5 April 1930 from the faeces of Susanna (*M. sinicus*), experimentally infected with Strain *D.* on 26 November 1929. Grown in Ehs medium (without starch) with addition of the bacteria accompanying Strain *K.*28c. Still under cultivation.

Strain DS.[2]—Isolated from the faeces of the same monkey (Susanna) on 8 April 1930, and grown in HSre + S medium. Abandoned voluntarily 30. iv. 30.

All these strains were very carefully tested before use. They were all morphologically typical; and in the process of isolation they were all caused to encyst *in vitro*, and continued by hatching the cysts (which were normal in every way) and propagating the resultant amoebae. The purity of these four strains was definitely ascertained before inoculation into kittens. They were all exactly alike morphologically (amoebae and cysts, at all stages), but differed in their origins, mode of cultivation, and concomitant bacteria.

[1] See Table XX, p. 50 *supra.*

[2] See Tables XVI, p. 45, and XVII, p. 46; also Scheme E, p. 48.

[3] I did this lest it might be objected that Strains *P.* and *DM.* had been isolated from their respective hosts too soon after their infection. The early isolation of these two strains was intentional, and was due to my fear that the monkeys in question might acquire other infections later through accidental contact with my other macaques. Susanna and Rosa, being the only monkeys which I had at the time of these experiments, were kept together in complete isolation throughout.

The four kittens (Nos. 43, 44, 45, 46) employed in this series of experiments were all very young at the time of inoculation—only 5 weeks old—and belonged to the same litter. They were under my daily personal observation throughout, and I examined (both culturally and microscopically) almost every stool passed by each. As all examinations were completely negative, I finally killed all 4 kittens and made a careful study of their intestines and intestinal contents *post mortem*. Not one of them showed any sign of infection with *E. histolytica*.

Although I have kept full records of these experiments, it seems unnecessary to reproduce them here. The final result was a decisive negative[1], and I therefore summarize only the relevant particulars in Table XXI.

Table XXI.

Kitten No.	Date of inoculation	Strain of amoebae*	Date of isolation	Source of strain	No. of negative examinations†	Result
43	28. iv. 30	*DR.* (8)	14. iv. 30	**Rosa** (*M. rhesus*) experimentally infected with Strain *D.* (*hominis*) 25. xi. 29	12	Negative. Normal till killed 19. v. 30. No amoebae or amoebic lesions found *post mortem*
44	28. iv. 30	*DRK.* (7)	14. iv. 30	Ditto	11	Ditto
45	29. iv. 30	*DS.* (13)	5. iv. 30	**Susanna** (*M. sinicus*) experimentally infected with Strain *D.* (*hominis*) 26. xi. 29	12	Negative. Normal till killed 20. v. 30. No amoebae or amoebic lesions found *post mortem*
46	29. iv. 30	*DS.*[2] (11)	8. iv. 30	Ditto	7	Ditto

* The figures in parenthesis denote the serial numbers of the subcultures used for injection.
† All examinations made both microscopically and culturally.

I have been able to make only a few experiments in this connexion, but they seem to show conclusively that a strain of *E. histolytica* which has lost its infectivity for kittens, as a result of continued cultivation *in vitro*, does not usually recover this faculty after subsequent passage through *M. sinicus* or *M. rhesus*. To make the six foregoing negative experiments clear, I have recapitulated them all, in a different form, in Table XXII. It must be emphasized, however, that all these experiments were made with derivatives of only one original strain of *E. histolytica hominis* and only one of *E. h. macacorum* (from *M. sinicus*). Other strains, from other hosts, might well have given different results. Nevertheless, in considering the facts recorded in this table it must also be remembered that both the strains employed had been shown to be originally infective for kittens, and both were proved to have lost this infectivity on cultivation, before they were introduced into macaques and then recovered in pure culture and tested on fresh kittens. The experiments, as far as they go, were conclusive; but they are obviously insufficient numerically to warrant any confident generalization.

[1] It will be seen that only 7 negative examinations are recorded for Kitten 46. This is because it passed faeces only 7 times during the whole of the experiment.

Table XXII.

Strain	Origin of Strain	Kitten inoculated	Result
P.	*M. sinicus* ⟶ Kitten 28 ⟶ *M. sinicus* ⟵ Strain *K.*28c. ⟶ Strain *P.*	No. 40	Completely negative
DM.	Man ⟶ Strain *D.* ⟶ *M. sinicus* ⟶ Strain *DM.*	No. 41	Ditto
DR.	Strain *D.* ⟶ *M. rhesus* ⟶ Strain *DR.*	No. 43	Ditto
DRK.	Strain *D.* ⟶ *M. rhesus* ⟶ Strain *DRK.*	No. 44	Ditto
DS.	Strain *D.* ⟶ *M. sinicus* ⟶ Strain *DS.*	No. 45	Ditto
DS.[2]	Strain *D.* ⟶ *M. sinicus* ⟶ Strain *DS.*[2]	No. 46	Ditto

(ii) *Some Observations on the Ingestion of Human red Blood-corpuscles by various Strains of* E. histolytica.

One of the best-known physiological peculiarities of *E. histolytica hominis* is its ability to ingest red blood-corpuscles—a character generally believed[1] to distinguish this species from all other intestinal amoebae of Man. I have therefore studied this character specially in all the strains previously described in the present paper, and the study has led to somewhat unexpected results. Some of these I must now describe, as they have a direct bearing upon the main problem here at issue.

[1] The belief is not well founded: for though it is true that no other species of *Entamoeba* living in Man will eat human red blood-corpuscles, it is not true that no other human intestinal amoeba possesses this power. *Dientamoeba fragilis* will also ingest human red corpuscles *in vitro*—as I have proved by experiment (unpublished). Moreover, as will appear presently, it is not true that all strains of *E. histolytica* eat red corpuscles.

Strains of *E. histolytica hominis*, recently isolated from patients suffering from amoebic dysentery, readily ingest human red blood-corpuscles when they are introduced into cultures. This is a fact familiar to all workers since it was first established by Boeck and Drbohlav (1925). Strain *D.*—repeatedly referred to previously—formed no exception to the rule: when first isolated the amoebae of this strain ingested red corpuscles with avidity[1]. But, to my surprise, all strains of *E. histolytica macacorum* which I isolated at the same time entirely refused to ingest human red corpuscles when tested in a similar manner[2]. This observation suggested that *E. h. hominis* and *E. h. macacorum* could be distinguished by this difference in behaviour; but further work has shown that this is not so. It is not possible to determine, by their ability or inability to eat human red blood-corpuscles *in vitro*, whether the amoebae in a given culture originated from a man or from a macaque. The character is not fixed but variable—a strain which possesses the power of ingesting corpuscles may lose it, and one which does not possess it may acquire it. But the data which I have hitherto collected are incomplete, and the problem is more complex than I originally anticipated; so I shall now record merely a few definite findings which are of importance for present purposes[3].

A strain of *E. histolytica* which ingests human red blood-corpuscles *in vitro* I shall call, in the present section, "positive"; and for the sake of brevity I shall refer to it as "*r.b.c.* +": and all strains which do not ingest human red blood-corpuscles I shall call "negative," and refer to as "*r.b.c.* −." But it must be noted that "positive" strains do not all ingest red corpuscles with equal avidity: they differ quantitatively. For example, if two different strains be tested simultaneously, in exactly the same way, it may be found after a given time that 50 per cent. (or more) of the amoebae in one culture contain red corpuscles, and only 5 per cent. (or less) in the other. Moreover, in the first many of the individuals may contain a dozen or more corpuscles, while those in the second never enclose more than one to two. But I have not yet been able to study this problem quantitatively, and therefore all the observations now recorded are merely qualitative (+ or −).

To ascertain whether a given strain of amoebae is positive or negative, it is usually only necessary to add a drop of fresh human blood[4] to a culture. The corpuscles rapidly sink to the bottom, and the amoebae—if able to do so—soon ingest them. A positive finding can often be registered very quickly (within 10–15 minutes); but with negative strains it may be necessary to examine the cultures repeatedly for several hours, in order to be certain of the result. As a rule there is no need to wash either the amoebae or the

[1] See Dobell and Laidlaw (1926*a*), p. 315.

[2] See Dobell and Laidlaw (1926*a*), p. 301.

[3] I hope to publish a fuller account of my researches in this connexion (covering other species besides *E. histolytica*) in a future instalment.

[4] In nearly all my experiments I have used my own blood: but such few tests as I have yet made with the blood-corpuscles of other persons or animals (monkeys and kittens) have given identical results.

corpuscles before making the test; but if such precautions be taken the result is identical—positive strains remaining positive, and negative strains negative. Some strains of *E. histolytica*, however, are accompanied by haemolytic bacteria, and when corpuscles are added to the cultures they dissolve so rapidly that no test is possible. With such strains, therefore, it is always necessary to wash the amoebae (to remove the excess of concomitant bacteria) before making the trial with red blood-corpuscles suspended in Ringer's fluid or other inert medium.

My chief findings may be briefly summarized as follows:

(*a*) It appears to be true that all strains of *E. h. hominis* recently recovered from human patients suffering from amoebic dysentery are *r.b.c.* +. (Strain *D.* furnishes a typical illustration.) It is not true, however, that all strains freshly isolated from Man are able to ingest human red blood-corpuscles *in vitro*; for my Strain *F.*, from a non-dysenteric subject[1], was from the first *r.b.c.* −. Both these strains, it will be remembered, readily infected kittens originally.

(*b*) All amoebae (*E. h. macacorum*) which I have yet isolated from Macaques directly, and tested *in vitro*, have been *r.b.c.* − and have remained so for as long as they were cultivated (several years in some cases). This statement applies to Strains *M.*4, *J.*, *Jc.*, and *T.* (from 3 individuals of *M. rhesus*), Strains *S.*, *B.*, *BX.*, *BC.*, and *M.* (from 3 *M. sinicus*), and Strain *Nc.* (from *M. nemestrinus*)[2]. Of these, one only (*B.*, from *M. sinicus*) was found to be infective to kittens: but though it thus resembled Strains *D.* and *F.* (*E. h. hominis*) in this character, it was a typical *E. h. macacorum* (*r.b.c.* −) in its behaviour towards human red blood-corpuscles.

(*c*) I have found that a strain of *E. histolytica* which is originally *r.b.c.* + may become *r.b.c.* − after prolonged propagation *in vitro*. Strain *D.* (cultivated continuously for 5½ years) was originally, as already noted, strongly *r.b.c.* +. It ingested red corpuscles with great avidity, and continued to do so, whenever tested, for nearly 2 years. The strain was isolated on 3. xi. 24 and was consistently *r.b.c.* + until 8. iv. 26. A further test on 29. x. 26 (200th serial subculture), however, though still *r.b.c.* +, was entered as "very poor": but on 23. ii. 27 (217th serial subculture) the result was completely negative. Since that date, Strain *D.* has consistently refused to engulf red corpuscles whenever tested. It has become, in this respect, indistinguishable from a typical *E. h. macacorum*[3].

(*d*) On the other hand, strains of *E. histolytica* which are originally *r.b.c.* − can become *r.b.c.* + after passage through a kitten. Strain *F.* (*hominis*), which

[1] See p. 50 *supra*.

[2] These strains are mentioned particularly because experiments already recorded were performed with most of them. Strains *T.* and *M.*, however, have not been previously described. The former was derived from Monkey No. 9, the latter from Mungo. (See Table I, p. 8.)

[3] It may be emphasized again here that infectivity for kittens, and ability to ingest human red corpuscles, are independent characters. They appear to be in no way correlated. Strain *D.* lost the former faculty—on continued cultivation—in about 2 months, but retained the latter for about 2 years. Cf. Dobell and Laidlaw (1926*a*), p. 315.

was *r.b.c.* −, infected[1] Kitten 24; and from this kitten I recovered two excellent substrains which were both *r.b.c.* +. Again, Strain *B.* (*macacorum*) was invariably *r.b.c.* −; but numerous substrains recovered from Kittens 15, 17, 18, 19, 20, and 21 (to which it was transmitted experimentally[2]) were *r.b.c.* +. They ingested human red blood-corpuscles quite as eagerly as Strain *D.*

(*e*) These findings suggest that the power to eat red corpuscles is correlated with the existence of dysentery in the host from which the strain (or substrain) of amoebae is derived. But this is not so: for I have found that amoebae which are strongly *r.b.c.* + may be cultivated from non-dysenteric hosts. For example, Strain *D.*, after it had become *r.b.c.* − (as a result of continued cultivation), was experimentally transmitted to *M. sinicus* (Mungo and Susanna) and *M. rhesus* (Rosa). None of these monkeys contracted dysentery, though all acquired heavy infections: but every substrain of Strain *D.* (*e.g.* Strains *DM.*, *DS.*, *DS.*[2], *DR.*, *DRK.*) recovered from these animals has proved to be *r.b.c.* +. Similarly, Strain *DM.* (derived from *D.*, after passage through *M. sinicus*) on prolonged cultivation lost its power of eating red corpuscles almost completely—like its parent. But after about 2 years *in vitro* it was experimentally transmitted to *M. rhesus* (Rosa), and when recovered again (Strain *DMR.*) from this monkey was found to be fully *r.b.c.* + once more.

An even more surprising phenomenon of the same sort has been observed in the case of Strain *K.*28c. This strain has always been *r.b.c.* −. It was derived from a kitten experimentally infected from *M. sinicus* (Bonar, all strains from whom were invariably *r.b.c.* −), and was isolated on the day when the kitten first showed dysenteric symptoms. During the last 4 years I have repeatedly tested this strain, and always with the same result: no amoeba has ever been seen to eat a single red corpuscle.

About 8 months after isolation, Strain *K.*28c. was experimentally introduced into *M. sinicus* again (Polo): but to my astonishment the substrains of *K.*28c. which I recovered in culture from this monkey were *r.b.c.* +. Some of them ingested red corpuscles excellently, though incapable of producing dysentery in *M. sinicus* or a kitten[3].

Although Strains *D.*, *DM.*, and *K.*28c. (which were originally, or had become, *r.b.c.* −) became *r.b.c.* + after sojourn in *M. sinicus* or *M. rhesus*, all natural strains obtained originally from the same monkeys were *r.b.c.* −. It thus seems impossible to suppose that the factor determining conversion from *r.b.c.* − to *r.b.c.* + was resident in the intestinal contents of the animals used in the experiments (*e.g.* the transformation cannot depend upon any individual peculiarity of the intestinal flora in certain monkeys).

(*f*) As already noted, a strain which is *r.b.c.* − may become *r.b.c.* + after passage through a kitten: for example, Strain *B.* (*r.b.c.* −) became *r.b.c.* + in all kittens infected with it (Nos. 15, 17, 18, 19, 20, 21). But I have found that the

[1] See p. 51 *supra.*

[2] Cf. Scheme A, p. 20 *supra.*

[3] See the experiment with Strain *P.* (which was *r.b.c.* +) and Kitten 40 (p. 52 *supra*).

transformation requires a certain time for its development. This is well illustrated in the case of Kitten 15, which was inoculated on 19. iii. 25 with Strain *B.* (*r.b.c.* −), and immediately became infected. A substrain of *B.* (*K.*15) recovered from Kitten 15 on 20. iii. 25 was, like its parent, *r.b.c.* −. But another substrain (*K.*²15), isolated 3 days later (23. iii. 25), was feebly *r.b.c.* +, and substrains recovered on 31. iii. 25 and afterwards were definitely positive. Similar observations were made on all other experimental kittens. It has already been noted, for example, that the first strain (*K.*28c.), isolated 6 days after infection, from Kitten 28 was *r.b.c.* −: but a second strain (*K.*²28), isolated from the same animal 12 days after infection, was *r.b.c.* +.

Similar findings have been recorded for all *r.b.c.* − strains which became *r.b.c.* + as a result of passage through macaques. The substrains recovered during the first few days after experimental infection have been almost invariably *r.b.c.* −, while those recovered later have been *r.b.c.* +. Transition from *r.b.c.* − to *r.b.c.* + in such experiments is never sudden, though it occurs very rapidly in comparison with the reverse transformation (*r.b.c.* + to *r.b.c.* −) effected by cultivation outside the body.

(*g*) In contrast with the experiments mentioned in (*e*) above—in which *E. h. hominis* (*r.b.c.* −) became *r.b.c.* + after passage through a macaque—are my observations on *r.b.c.* − strains of *E. h. macacorum*. No strain of simian origin has ever become *r.b.c.* + after passage solely through another simian host. Examples of this are seen[1] in the history of Strains *Nc.* and *Jc.* The former was obtained from *nemestrinus*, transmitted then to *rhesus*, and thence to *sinicus*. The strains from all these animals (*Nc.*, *NRK.*, *NRS.*, etc.) were all consistently *r.b.c.* −. Similarly, Strain *Jc.* (from *rhesus*), after passage through *sinicus* (Strain *JSc.*), remained *r.b.c.* − throughout.

The contrast in the behaviour of *r.b.c.* − strains of *E. h. hominis* and *E. h. macacorum* is particularly striking in the case of Strains *D.* and *Nc.*, because both were experimentally transmitted, with opposite results, through the same monkeys. Strain *Nc.* (a *nemestrinus*-strain, *r.b.c.* −) was transmitted to Rosa (*M. rhesus*) and thence to Susanna (*M. sinicus*). All substrains recovered from both these hosts were equally *r.b.c.* −. On the other hand, Strain *D.* (which had become *r.b.c.* − on cultivation) completely regained its power of ingesting red corpuscles after passage through the very same two macaques.

Although I have made many other observations in the present connexion[2], I am still unable to reduce the data to general principles. Many of the facts are perplexing and some of them appear contradictory; but they show clearly that ability to ingest human red blood-corpuscles *in vitro* is a fluctuating physiological character in *E. histolytica*, and consequently that it has no systematic value. It is at least certain that strains possessing this power may

[1] Cf. Scheme D, p. 39, and Scheme C, p. 36.

[2] Kessel (1928*a*, p. 285) also appears to have made some observations similar to mine, but they are not recorded in sufficient detail for comparison. His belief that *r.b.c.* − amoebae can be made *r.b.c.* + by merely transplanting them into a less rich medium "overnight," is in complete disagreement with my experience.

lose it, and again recover it; and that others which possess it not may acquire it—though the determining factors in both cases are still obscure.

SUMMARY OF THE PRECEDING SECTION (pp. 49–60).

(i) The fact has been confirmed that strains of *E. histolytica* which, when newly isolated, are infective for kittens, may lose this infectivity after continued cultivation *in vitro*.

A strain (*F.*) of *E. h. hominis*, isolated from a non-dysenteric subject, was found to be capable of infecting a kitten quite as readily as another strain (*D.*) isolated from a patient with acute amoebic dysentery.

When strains of *E. h. hominis* and *E. h. macacorum* have lost their infectivity for kittens (through cultivation *in vitro*), it has been found impossible to restore it by subsequent passage through *M. sinicus* or *M. rhesus* (six experiments, summarized in Table XXII, p. 55).

(ii) This subsection—being itself a summary of various isolated observations and experiments—cannot be recapitulated in few words. The recorded data demonstrate, however, that ability or inability to ingest human red blood-corpuscles *in vitro* is an inconstant character of *E. histolytica* (both *hominis* and *macacorum*). In certain circumstances, strains which possess the power may lose it, and those which do not possess it may acquire it.

IV. DISCUSSION.

I shall make this section as short as possible, and confine my remarks to matters of observed fact and actual experiment. In my opinion the time is not yet ripe for discussion of all the wider problems involved[1].

So far as I have been able to ascertain—and I have searched the literature of the last 50 years carefully—only two other workers have tried to infect kittens with *E. histolytica macacorum*, and only one[2] has made a serious attempt to transmit *E. h. hominis* to any species of *Macacus*. Before proceeding further I shall briefly discuss their findings.

The first attempt to infect kittens with *E. histolytica* from macaques was made by Mello (1923)[3], who records that he injected "dysenteric faeces" from *M. sinicus*—apparently containing amoebae of *E. h. macacorum*—intrarectally into two kittens, which developed dysentery 15 and 19 days later respectively. Both showed numerous amoebae in their dejecta, and in both the dysenteric

[1] A tabular summary of earlier work on the intestinal protozoa of monkeys has recently been published by Kessel (1928*a*, pp. 276–278). Unfortunately his tabulation is incomplete, and contains not a few errors.

[2] I leave out of account, for the moment, the negative experiments performed by Walker (1913) on "monkeys" of unrecorded species (see p. 68 *infra*).

[3] From Mello's paper we learn that his experiments were made in 1913, in Laveran's laboratory at the Institut Pasteur (Paris): but he worked up his material at Turin after the War, and did not publish his findings until 1923. Kessel (1928*a*, p. 277) overlooks the fact that Mello records the species of *Macacus* which he employed, and erroneously states that his monkeys were studied in Italy.

symptoms progressed for 2 months: but at this stage Mello unfortunately abandoned his kittens—believing their deaths to be imminent. The final result of his experiments is therefore unrecorded and apparently unknown.

All that I can conclude from this imperfectly published[1] work is that Mello inoculated 2 kittens with *E. h. macacorum* from *M. sinicus*, and produced in them a slowly developing and chronic—rather than acute—form of amoebic dysentery. (Though the author himself does not mention it, an incubation-period of 15–19 days, followed by survival for 2 months, is most unusual in similar experiments with *E. h. hominis*.) Mello's observations, as far as they go, are thus in harmony with my own results obtained with Strain *B.*, from the same species of macaque[2]: and to Mello, accordingly, belongs the credit of having first shown that the *histolytica*-like species of *Entamoeba* found in *M. sinicus* is transmissible to kittens.

Three years after the appearance of Mello's paper, Kessel (1926) announced[3] that he had succeeded in infecting 2 cats by means of "rectal injections of monkey feces containing cysts of the dysentery-like [*sic*] amoebae of a naturally infected monkey" [no species recorded, but from later accounts apparently *M. rhesus*]. A fuller description of these and other experiments appeared subsequently (Kessel, 1928), and must be considered in detail.

Kessel's experiments—owing to the way they are recorded—are not very easy to follow. So far as I can discover from his two later papers (1928, 1928 *a*), all his work was done in Peking with mixtures of cysts in faeces—not with pure strains in cultures—and he tested such material from only 3 monkeys. (I leave out of account his undiscussible experiments with unknown mixtures of amoebae from undetermined species of "monkeys in Zoological Gardens"—his Series II.) The monkeys were all macaques, but of two different species—2 *M. rhesus* (Nos. 12 and 16) and 1 *M. sancti-johannis* (No. 8). I shall consider his results separately, in order to compare them with my own. His actual findings were apparently as follows:

(1) Cysts of *E. h. macacorum* in faeces of *M. rhesus* (No. 12) were administered *per os* to 4 kittens (Nos. 111, 112, 113, 114). Two contracted amoebic dysentery, two did not (Series I).

(2) Similar cysts in the faeces of another *M. rhesus* (No. 16) were inoculated *per anum* into 3 other kittens (Nos. 118, 119, 120). Two became infected (no dysentery observed) and 1 did not (Series III). From one of the infected kittens (No. 118) the amoebae were recovered in culture and injected *per anum* into 2 further kittens (Nos. 121, 122). Result: 1 positive, 1 negative (Series IV).

[1] Not only is Mello's description incomplete, but his whole paper bristles with misprints and other errors.

[2] Cf. p. 19 *supra*.

[3] Kessel's experiments are not dated in his full account, but appear to have been performed at about the same time as my own with Strain *B*. A preliminary notice of these was published in the annual *Report of the Medical Research Council* for 1924–25, which was issued in January 1926 (though dated 1925). Kessel's preliminary paper appeared later in the same year (May, 1926) and his final account in 1928. The only full description of my own researches is contained in the present paper. I note these dates in order to aid future historians.

(3) Cysts of *E. h. macacorum* in the faeces of *M. sancti-johannis* (No. 8) were given *per os* to 2 kittens (Nos. 123, 124). Both became infected (Series V).

To the best of my knowledge these are the only experiments ever made which show that *E. histolytica* from *Macacus rhesus* and *M. sancti-johannis* can parasitize kittens. They are therefore of special interest in relation to the observations of Mello and myself on *E. histolytica* from *M. sinicus*[1].

Kessel does not stress the point, but I would note here that in his 7 attempts with faecal cysts from *M. rhesus* (2 individuals), he succeeded in infecting only 4 kittens. Three kittens (Nos. 111, 112, 119) acquired no infection, though inoculated in the same way. I attach importance to these negative results, because I have found that some strains of *E. h. macacorum* from *M. rhesus* are non-infective to kittens[2].

Kessel believes that there is no essential difference[3] between the amoebic dysentery typically produced in kittens by *E. h. macacorum* and that produced by *E. h. hominis*, though he allows that there is "a slightly longer incubation period and a longer interval between the time of infection and death[4]" with the former. From his own results he concludes that, with *E. h. macacorum*, "the incubation period is ten instead of six days and the average time elapsing between infection and death is fourteen instead of nine days, which are the corresponding periods in kittens infected with human amoebae[5]." Everybody will agree that the "incubation period" is usually very short, and death seldom long delayed, in kittens infected with *E. h. hominis*: but I have found the incubation period—or latent period, as I prefer to call it—equally short with *E. h. macacorum*, though I have found that the dysentery is less acute and less rapidly fatal. (All my kittens were killed, so that their "time of death" was arbitrarily fixed: but one lived for 10 weeks, and all of them for more than 14 days; while two recovered spontaneously, after remaining infected for at least a fortnight.) But Kessel's own records do not appear to support his estimates of 10 and 14 days for the average "incubation period" and "time of survival" respectively.

I do not know how Kessel determined the "incubation period" for his kittens, but he does not seem to have made careful microscopic and cultural examinations of their dejecta every day—the only sure method. Moreover, he records the "incubation period" for only 3 animals—No. 121 (12 days), No. 123 (6 days), and No. 124 (18 days). Two other kittens, however, Nos. 118 and 120, which were infected successfully, appear to have remained healthy

[1] All my own experiments with *E. histolytica* from *M. rhesus* (only one individual) were completely negative; and nobody but Kessel seems yet to have studied the amoebae of *M. sancti-johannis*.

[2] It should be remembered that Kessel's work was not done with pure strains, but with unknown mixtures of amoebae and cysts in faeces.

[3] I agree with this: but I believe there *is* a difference, though it is one of degree and not of kind.

[4] Kessel (1928*a*), p. 283.

[5] Kessel (1928), p. 325.

for no less than 62 days[1], when they were killed. Presumably the "incubation period" of the disease would have been more than 62 days, therefore, in these animals if they had not been sacrificed. Consequently, Kessel's figure (10 days) for the average "incubation period" can hardly be accepted without further evidence.

As regards the figure given for the average time of survival after infection, I also find difficulty in discovering how it was arrived at. Kessel gives it as 14 days: but at least two of his kittens (Nos. 118 and 120, just mentioned) lived for 62 days, when they did not die but were killed, and were both found to be "apparently healthy at autopsy." Again, in Kessel's "Series I" it is seen that 2 kittens (Nos. 113, 114) died or were killed on the 15th and 12th days of the disease respectively: but 2 other kittens similarly inoculated (Nos. 111, 112) also died on the 17th and 15th days without becoming infected. This control experiment—though not recorded as such—obviously robs the dates of death of Kittens 113 and 114 of all evidential value.

If all Kessel's findings—so far as they have been reported—be considered, they do not seem to disagree with Mello's or mine. On the contrary, they seem conformable with the view that kittens infected artificially with *E. h. macacorum* usually display a less acute form of amoebic dysentery than those infected with *E. h. hominis*, and that some strains of the parasite from macaques are even non-infective.

The only evidence—besides my own—which seems to show that *E. histolytica hominis* can be transmitted to macaques[2] has also been furnished by Kessel. His experiments were somewhat complicated, however, and his account of them unfortunately omits many essential particulars.

Kessel (1928*a*) apparently made only two experiments[3], and they were both made with mixtures of intestinal protozoa contained in human faeces. He did not use pure strains of *E. histolytica*. According to his account, he desired to repeat his earlier work (with species other than *E. histolytica*) "with monkeys negative for Protozoan infection. None were procurable, but four young monkeys were given yatren in an attempt to free them of their intestinal Protozoa. Apparently, excessive dosage was given at first and two of the monkeys weakened and died of pneumonia during treatment. Two monkeys, Nos. 13 and 14, survived treatment and each was examined twenty-eight times between

[1] I cannot believe that the infections in these cats could have remained undiscovered for so long a time if they had been subjected to careful examination every day—microscopically and culturally—during the whole of this period. Kessel, however, only discovered their infections at necropsy.

[2] Experiments such as those recorded by Franchini (1912), Ujihara (1914), and Mayer (1919) hardly seem worth discussion, as they were probably performed with monkeys already infected naturally with *E. histolytica*. At all events, these authors give no evidence to prove that they were not.

[3] I presume that the "two monkeys found to be amoeba-free," mentioned by Kessel (1926) in an earlier note, were identical with those described later (Kessel, 1928*a*), though there are slight discrepancies between the two accounts.

January 28 and April 20, 1925, following the treatment. Monkey No. 13 was entirely freed of intestinal Protozoa and monkey No. 14 showed a very light infection of *Iodamoeba* only" (Kessel, 1928*a*, p. 295). Nothing further is recorded about these treatments; but it appears from Tables 1 and 4 that Monkeys 13 and 14 were both *M. rhesus*, and that they both originally harboured *E. histolytica* and *Endolimax nana*, while No. 14 was also infected with *E. coli* and *Iodamoeba*. It will thus be seen that Kessel claims to have freed his two macaques not only of *E. histolytica*, but also of *E. coli* and *E. nana*. It is regrettable, therefore, that he gives no details of his methods[1]. The evidence that Monkeys 13 and 14 were cured of their infections with *E. histolytica macacorum* appears to be strong—28 negative examinations in 82 days following treatment: but it would have been much stronger if the examinations had been made daily, and if we had been assured that Kessel himself made them (culturally as well as microscopically).

The account of the experiment continues: "Both monkeys were fed the intestinal Protozoa indicated in the table, monkey No. 13 being given three feedings on April 22, 23, and 25, respectively, and monkey No. 14 only one feeding on April 21." (Table 4 shows that No. 13 was fed on *E. histolytica*, *E. coli*, *Iodamoeba*, and *Chilomastix*, and No. 14 on *E. histolytica*, *E. coli*, *E. nana*, and *Chilomastix*.) It is added that "the human feces containing the Protozoa were diluted with water and then given by mouth": and I assume, therefore, that "the Protozoa" means the *cysts* of all the species mentioned, but this is not stated. The result of the experiment was that "cysts [no species indicated] first appeared in the stool of monkey No. 14 on May 2, twelve days [11, according to my reckoning] after the first feeding, and on May 11 in the feces of monkey no. 13, twenty-one days [19?] after the feeding. Subsequent examination of the feces at intervals until July 6 showed that *E. dysenteriae* [= *E. histolytica*], *E. coli*, *Iodamoeba*, and *Chilomastix* had been established in monkey No. 13 and *E. dysenteriae*, *E. coli*, and *E. nana* had been established in monkey no. 14." As the examinations do not appear to have been made daily and as it is not stated when *E. histolytica* was first found in the faeces of either monkey, I cannot tell whether Kessel's findings agree with mine or not—save in so far as they indicate that *E. histolytica hominis* is transmissible to *M. rhesus*.

Kessel's later observations on Monkeys 13 and 14, however, are entirely consistent with my own. "No dysenteric symptoms were present at any time during the two months[2] before the monkeys were autopsied. On July 6 both monkeys were sacrificed. Cysts and trophozoites of *E. dysenteriae* were re-

[1] As far as I am aware no other worker has yet studied the action of yatren on amoebic infections in any monkeys; and though great pretensions have been made for this drug as a cure for *E. histolytica* infections in man, even the German enthusiasts do not claim that it is also a specific remedy for *E. coli* and *E. nana*. It is to be hoped, therefore, that Kessel will publish further particulars regarding the weights of his animals, dosage, duration of treatment, mode of administration, etc., with details of his methods of examination and actual findings.

[2] This is referred to as "a period of three months" in Kessel's preliminary paper (1926). The actual time appears to have been from April 21 or 22 to July 6.

covered from the intestinal lumen and crypts...but no definite pathological lesions were found." (Unfortunately, nothing is said about the clinical condition of the human subjects from whom the strains of *E. histolytica* used were derived.)

It thus appears that Kessel probably succeeded in infecting two *M. rhesus* with *E. histolytica hominis*, without producing dysentery or lesions in either. As his results are in complete agreement with the expectation from my own findings, I am prepared to accept them—though I cannot regard his evidence, so far as he has yet presented it, as in itself conclusive. He would obviously have obtained the same final results if he had not (as he believed) cured his macaques of their original infections with *E. h. macacorum*, and if his experimental feeding with human faeces had really been non-infective.

Several workers[1] have been unwilling to believe that a strain of *E. histolytica* may lose its infectivity for kittens as a result of continued cultivation. Since my first observations[2] were published, however, I have confirmed them; and I hope the evidence given in the present paper will carry conviction. I am personally satisfied that some strains of *E. histolytica* (both *hominis* and *macacorum*) may become completely non-infective for kittens if cultivated for a sufficient time *in vitro*. I have no idea *how* this change is brought about, or what factors—beyond artificial cultivation, and all that it implies—determine loss of infectivity and pathogenicity[3]: but I regard the transformation as a fact, and an important one.

After this brief consideration of certain data about which, at present, there is some legitimate difference of opinion, I must discuss the bearing of the data as a whole on the main problem at issue. I can do this most clearly by means of a comparison.

If we compare a typical strain of *E. histolytica hominis*, in a man suffering from acute amoebic dysentery, with a typical strain of *E. h. macacorum* living in a healthy macaque, we note at once certain resemblances and certain differences. Morphologically the parasites from both hosts are essentially identical at every stage in their life-histories[4]: under the same conditions they develop in the same way *in vitro*[5]: and they can both be eradicated by treating

[1] For example Sautet (1926) and Rees (1928). The former, however, has since confirmed my observations (see Sautet, 1929), as also has Sanders (1928).

[2] See Dobell and Laidlaw (1926*a*), p. 314.

[3] Sautet (1929) and Rees (1928) apparently credit me with the belief that it is only necessary to feed any strain of *E. histolytica* on starch to make it promptly non-infective for kittens: and Chiba and Kuwabara (1930) even credit Dr Laidlaw and myself with an experiment showing that starch is the determining factor. But we never performed this experiment, and have never held the views attributed to us. We contented ourselves with recording that one strain of *E. h. hominis* had lost its infectivity after continued cultivation in a starch-containing medium which we devised, and never suggested that the mere addition of starch to any medium would cause all other strains to lose their infectivity likewise.

[4] Cf. Dobell (1919, 1928).

[5] Dobell (1928).

their hosts with emetine[1], and are equally sensitive to minute amounts of this alkaloid in cultures[2]. The chief differences are: (1) *E. h. hominis* (in the case postulated) lives as a parasite in the tissues of its host—ingesting his red blood-corpuscles, when available, both *in vivo* and *in vitro*; *E. h. macacorum* lives as a harmless commensal in the lumen of the gut—not attacking the tissues or ingesting corpuscles, but feeding upon bacteria and faecal debris. When tested *in vitro*, moreover, it usually appears to be unable to eat human blood-corpuscles. (2) *E. h. hominis* is usually able to parasitize kittens, producing acute and rapidly fatal dysentery: *E. h. macacorum* is frequently incapable of infecting kittens, and, when it does so, produces a milder or chronic form of dysentery from which the animal may recover spontaneously.

When we come to study these differential characters more closely, however, we find that they are not constant. *E. h. hominis*, when grown *in vitro*, ingests bacteria, starch-grains, etc., just like *E. h. macacorum*. On continued cultivation it may lose its power of infecting kittens; and on still more prolonged maintenance *in vitro* it may also lose its power of ingesting human red blood-corpuscles. A typical *E. h. hominis*, therefore, may become indistinguishable from a typical *E. h. macacorum*. This transformation actually occurred in the case of my Strain *D.*, which was derived from a man suffering from acute amoebic dysentery, and was originally capable of causing a similar disease in a kitten. It also, at first, ingested human red blood-corpuscles with avidity in cultures. After cultivation for 5 years, however, during which time it had lived chiefly upon a diet of bacteria and starch, and had never been in contact with living tissue, it completely lost its ability to parasitize kittens and to ingest human red corpuscles. But was this modified strain of *E. h. hominis* really indistinguishable from a typical *E. h. macacorum*? One most important character had still to be determined: could it actually live in a macaque? The experiment was tried, and was completely successful. Strain *D.*, after losing its power of infecting kittens and ingesting blood-corpuscles was introduced by the natural route (cysts *per os*) into 2 macaques of different species, in which it produced heavy infections—indistinguishable from their original natural infections with *E. h. macacorum*—which have now persisted for over 6 months.

Every other physiological character which appears at first sight to distinguish *E. h. hominis* from *E. h. macacorum* is found likewise to be inconstant. It would be wearisome to repeat all the evidence here, but the following points must be recalled: (1) Some workers (Gupta, 1925; Knowles, 1926; Kessel, 1928*a*) have found that strains of *E. h. macacorum* isolated directly from *M. rhesus* are able to ingest human red corpuscles *in vitro*[3]—like a typical

[1] Dobell with Bishop (1929).

[2] Cf. Dobell and Laidlaw (1926); Laidlaw, Dobell, and Bishop (1928).

[3] This is contrary to my experience; but the *r.b.c.* + strains were probably all derived from macaques suffering from dysentery, while all the strains which I have studied were from healthy animals.

E. h. hominis. (2) There is evidence, though it is as yet by no means conclusive, that *E. h. macacorum* can cause amoebic dysentery in macaques (McCarrison, 1921; Mello, 1923; Kessel, 1928*a*, etc.). (3) Although they are rare, cases are on record in which *E. h. hominis* has caused chronic amoebic dysentery in kittens—not the typical acute form of the disease. Knowles (1925), for example, has studied a kitten which remained alive and grew, showing only intermittent symptoms, for over 2 months after infection with *E. h. hominis*; while Sanders (1928) mentions a similar cat which remained infected for 2 months, and then recovered spontaneously. (4) *E. h. macacorum* appears[1] to be able to cause hepatic abscess in macaques (Castellani, 1908)—just as *E. h. hominis* occasionally does in human beings. (5) It appears almost certain now that *E. h. hominis* sometimes lives in men as a harmless commensal —just as *E. h. macacorum* typically does in its natural hosts. (Cf. Reichenow, 1926; Knowles, 1926*a*; Kessel, 1928*a*, etc.)

There is still, however, one important property of *E. histolytica macacorum* about which we have no information. Although it has been shown that *E. h. hominis* can parasitize Macaques (of two different species), the converse experiment has never been made—for obvious reasons. Nobody has yet proved experimentally that *E. h. macacorum* can infect Man. Nevertheless, from what we already know it is hardly possible to believe that such an experiment—if properly performed—would not succeed.

All the evidence yet obtained thus shows clearly that "the *histolytica*-like *Entamoeba* living naturally in Macaques" is of the very same species as the similar parasite of Man. It is, in fact, *E. histolytica*, and should therefore be known in future by this name alone. Owing to the imperfections of published records, however, it is by no means easy to determine how many species of *Macacus* (= *Silenus*) are naturally infected with *E. histolytica*. Perhaps all species are; for there are more or less certain indications for at least nine. *E. histolytica*, or an organism not at present distinguishable from this amoeba, has already been recorded from the following members of the genus[2]:

Species of *Macacus* [= *Silenus*]	Recorder
(1) *M. rhesus*	? Noc (1909); Mathis (1913); ? Swellengrebel (1914); Behrend (1914); Dobell (1919); Mello (1923); Bach (1923); Gupta (1925); Dobell and Laidlaw (1926*a*); Knowles (1928); Kessel (1928*a*); Dobell (1928); Faust (1930)
(2) *M. sinicus* [= *radiatus*]	Chatton (1912); McCarrison (1921); Mello (1923); Dobell and Laidlaw (1926*a*); Dobell (1928)
(3) *M. nemestrinus* ...	Dobell (1928)
(4) *M. cynomolgus* [= *irus*]	Musgrave and Clegg (1904); Mello (1923); Brug (1923); Kessel (1928*a*)

[1] Further evidence on this head is needed. Up to the present, liver-abscess due to *E. h. macacorum* has not been produced experimentally in any animal.

[2] I may remind the reader again that my nomenclature is that of Forbes (1897).

Species of *Macacus* [= *Silenus*]	Recorder
(5) *M. pileatus* [= *sinicus*]	Castellani (1908); Hirst [*in litt.**]
(6) *M. philippinensis* ...	Musgrave and Clegg (1904)
(7) *M. tcheliensis*† ...	? Noc (1909); Mathis (1913)
(8) *M. lasiotis*	Kessel (1928*a*)
(9) *M. sancti-johannis* ...	Kessel (1928*a*)

* Dr L. F. Hirst informs me in a personal communication that he has found "*E. histolytica*" in the intestine in this species.

† Considered by some specialists to be merely a subspecies or variety of *M. lasiotis*.

This list, though incomplete, shows how widely distributed *E. histolytica* must be among the various Macaques in different parts of Asia (India, Ceylon, Tonkin, Malaya, Java, China, and the Philippines[1]). That at least four species (*M. rhesus*, *M. sinicus*, *M. nemestrinus*, *M. sancti-johannis*) harbour *E. histolytica* seems certain; while the observations made on *M. pileatus* (which is very closely related to *M. sinicus*), and on *M. lasiotis* and *M. tcheliensis* (both closely allied to *M. rhesus*) indicate, by analogy, that these species are also infected: and one can hardly suppose that the very similar amoebae in *M. cynomolgus* and *M. philippinensis* (two closely related species) are specifically different from those in *M. sinicus*, *M. rhesus*, and *M. nemestrinus*.

Though most workers who have studied any macaques carefully have had no difficulty in finding *E. histolytica* in their faeces, and in infecting them experimentally with the human parasite, there are some negative records which ought not to be ignored. Walker (1913) made repeated attempts to infect two "monkeys" [? *M. philippinensis*] with *E. histolytica hominis*, but with entire lack of success. These apparently careful and thorough experiments[2] are somewhat surprising, as they seem to show not only that Walker's monkeys were uninfectible with *E. h. hominis* but also that they were naturally uninfected with *E. h. macacorum*[3]. In the same category may be included some recent observations by Deschiens (1927), who carefully examined 9 individuals of *M. sinicus* without discovering *E. histolytica* in any of them.

[1] It seems probable—from various indications in the literature—that the Japanese and Formosan monkeys (*M. fuscatus* [= *speciosus*] and *M. cyclopis*) are also infected, but I have been able to discover no definite records for these species.

[2] "In these experiments 2 monkeys were fed repeatedly with faeces containing encysted *Entamoeba histolytica*; 1 monkey was fed a dysenteric stool; 1 monkey received, *per oesophagus* [*sic*] after washing out the stomach with a suspension of magnesium oxide, a bloody mucous stool by stomach tube; 1 monkey received an injection of dysenteric stool into the rectum; 1 monkey received an injection of dysenteric stool into the caecum; and 1 monkey received an inoculation of entamoebic liver-abscess pus into the liver" (Walker, 1913, p. 304).

[3] Kessel (1928*a*, p. 293) says "Dobell (1917) records two unsuccessful attempts on his own part to infect two monkeys (*M. rhesus*)" with *E. histolytica*: but this is not correct. The attempts (which I mentioned in 1919) were to produce *amoebic dysentery*—nothing else—in these two macaques, by inoculation with *E. h. hominis* from a kitten. As both monkeys already harboured *E. h. macacorum* before the experiment, it was impossible to determine whether they became *infected* or not. This work was done jointly with Dr H. H. Dale.

I have taken it for granted that the amoebae found in my own macaques, and in those studied by others, were indigenous in their respective hosts. As most monkeys hitherto investigated have been captive animals, however, it might be suggested that their infections were not natural. The question has, indeed, already been raised by Kessel (1928*a*, p. 279), who remarks that "there is no positive assurance that the infections were acquired in the natural state before captivity, there being always the possibility that the infections were of human origin." I admit this possibility, but regard it as remote. It seems most unlikely that all the many macaques already found to harbour *E. histolytica* should have acquired their infections from human beings—with whom they must all have been in less intimate contact than with their own kindred. Moreover, macaques appear to be far more frequently infected with *E. histolytica* than human beings, so that it is more probable that human infections are occasionally contracted through contact with macaques than that captive macaques have all acquired their amoebae from human beings. If Kessel's suggestion should prove to be well founded, all the work which he and I have done to show that *E. h. hominis* and *E. h. macacorum* are identical will appear superfluous; but the main conclusion of the present paper will, on the other hand, be amply confirmed[1].

If it be accepted that "*E. histolytica macacorum*" and "*E. h. hominis*" are the same species, then the possibility that human beings may contract amoebic dysentery through accidental infection from macaques must be considered. Hirst (1929) has already raised this question, and has realized the importance of protecting water-supplies in the tropics from pollution by monkeys: but this is a problem which cannot be discussed here.

A less practical and more academic problem must finally be considered. Separate specific names have been proposed for the *histolytica*-like amoebae from some of the species of *Macacus* listed above. All these must now, I think, be regarded as invalid. *Entamoeba nuttalli* Castellani, 1908; *E. duboscqi* Mathis, 1913; and probably *E. chattoni* Swellengrebel, 1914; *E. cynomolgi* Brug, 1923; and *E. sinici* Brug, 1923 [*non* Chatton, 1912], must henceforth fall under the synonymy of *E. histolytica*. It should not be forgotten, however, that all these names have been proposed for "species" about which we still have little accurate information.

Although I am satisfied that certain species of *Macacus* are naturally infected with *E. histolytica*, I cannot follow those authors who indulge in wider systematic generalizations. From the scanty facts at present known, it seems to me unwarrantable to assume that all *histolytica*-like amoebae in all monkeys are identical. Obviously it is not yet justifiable to infer, from what little we know about the amoebae of the Macaques, that all Cercopithecidae harbour

[1] A decisive answer to Kessel's objection can only be obtained by a study of wild macaques. I know of no published record of the findings from such animals; but Dr L. F. Hirst has informed me privately that he found amoebae and cysts indistinguishable from *E. histolytica* in a wild *M. pileatus* shot in the jungle in Ceylon.

E. histolytica: and still less justifiable can it be to conclude that this species is common to all the Catarrhines. Those writers who, apparently regarding all "monkeys" as equal, go even further and include the amoebae of the Platyrrhines[1] in their all-embracing survey, undoubtedly trespass beyond the limits of legitimate scientific inference. It cannot be too strongly emphasized, at the present moment, that the Primates are many and various, and that their *Entamoebae* are still, as a whole, almost unstudied and unknown. Every species of *Entamoeba* in every simian host must be investigated in detail—by all the laborious methods of modern protozoology—before final conclusions can be drawn regarding its systematic status and its kinship with the intestinal amoebae of Man.

General Conclusions.

The main conclusion to be drawn from all the experiments here described[2] is that the *histolytica*-like *Entamoeba* living naturally in *Macacus rhesus*, *M. sinicus*, and *M. nemestrinus* (and probably in other species of the genus) is identical with *E. histolytica* of Man. All the evidence hitherto obtained supports this interpretation, and no sound arguments can be urged against it.

Other conclusions which can be drawn from all my researches in the present connexion—including those published previously—are of more general interest. It has been shown that monkeys of the genus *Macacus* (= *Silenus*) are natural hosts of *E. histolytica*, and can therefore be used instead of human beings for experimental study of this parasite. It has further been shown that it is now practicable (1) to isolate any desired strain of *E. histolytica* from a man or a macaque, and maintain it *in vitro* for any required period: (2) to obtain at will any stage in its life-history *in vitro* at any given moment: (3) to introduce this strain into a natural host (*Macacus*), study its behaviour therein, and recover it in culture whenever necessary: and (4) to eradicate it from this host permanently, if desired, by chemotherapeutic methods. Host and parasite can thus be kept separate, can be united, and can again be separated *ad libitum*; and any desired experiments can therefore be performed with either severally or with both combined. I believe that we possess, at present, such complete experimental control over no other animal endoparasite.

[1] As an example, I may note that Kessel (1928*a*) does not hesitate to include the very dubious and imperfectly studied amoebae of *Ateles* within his purview of *E. histolytica*. Like many other writers, also, he frequently refers to "the monkey" as though there were but one species of non-human Primate.

[2] Separate summaries of each series of experiments are given on pp. 30, 40, 49, and 60, so there is no need to resummarize my findings here. If the reader will turn to these pages, and then to the Discussion on p. 60, he will be able to appreciate the extent and variety of the evidence without wading through all the details.

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[1] The word is misprinted "protozoloögische" in the original.

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DIGESTION IN *CHRYSOPS SILACEA* AUST. (DIPTERA, TABANIDAE).

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Chrysops silacea is well known as an intermediate host of *Loa loa*, the causative organism of Calabar swelling, in West Africa (Leiper, 1913; Connal, A. and S. 1922); and as such the chemistry of its digestion must be of some interest to parasitologists.

The material on which this account is based was collected in the neighbourhood of Sapele, to the west of the Niger delta, in June 1928, during a research tour made on behalf of the London School of Hygiene and Tropical Medicine. The observations are necessarily incomplete, for I was unable to spend more than four days in the locality; and it was only by the great kindness and assistance of Dr S. L. D. G. Maclaine, of the West African Medical Service, and Mr A. B. Marr, Manager of Messrs Miller Brothers' rubber plantations, who very kindly allowed me to make use of the tappers on the estate, that I was able, in this short time, to do the work at all, and to obtain for dissection some three hundred flies.

The distribution of *Chrysops silacea*, its habits, manner of feeding, the effects of its bite and so forth, have already been described in full by Connal, A. and S. (1922); and I am much indebted to Dr and Mrs Connal for the information which they gave me on these matters.

Anatomy and histology of the intestine.

Dissections of the fly, and serial sections of the gut at various times after feeding, gave results which agree exactly with those of Cragg (1920) on *Tabanus*, both as regards the changes in the secretory cells of the epithelium and the changes in the ingested blood. It is unnecessary, therefore, to repeat these descriptions. One or two points, only, not mentioned by Cragg may be noted.

The "oesophageal valve." The anterior end of the mid-gut is dilated, and the lower end of the oesophagus is invaginated into it. But compared with that in most insects the invagination is slight; and this is associated, no doubt, with the absence of a peritrophic membrane; for the "oesophageal valve" of insects, when fully developed, seems always to be part of a mechanism for producing the peritrophic membrane (see Wigglesworth, 1930).

The "mesenteric sphincter." At the hind end of the mid-gut, immediately above the entry of the Malpighian tubes, there is a valve or sphincter just like that described in *Glossina* by Lester and Lloyd (1928).

Entry of blood into the tracheae. After a large meal which stretches greatly the wall of the gut, some of the blood may enter the tracheae and form there black threads of haematin like those already described in *Glossina* (Wigglesworth, 1929), though never so extensive. These threads assume many forms, and in some cases they are so like "Ross's black spores" in mosquitoes as to suggest that some at least of these may have a similar origin (cf. Mayne, 1929).

Functions of the parts of the digestive system.

Digestive enzymes. The methods employed for studying the digestive enzymes were the same as those already described (Wigglesworth, 1927, 1928, 1929). The salivary glands, the "cardia" (the narrow anterior half of the mid-gut (see Cragg, 1920)) and the "stomach" (the pyriform posterior half of the mid-gut) were examined separately. The results are given in Table I; and the results obtained previously in *Glossina* and *Calliphora* are given, for comparison, in Tables II and III. The + signs indicate roughly the relative activity of the various enzymes, the sign ± signifying definite but very weak activity.

It will be seen that the findings in *Chrysops* are intermediate between those in *Calliphora* and *Glossina*. In *Calliphora*, enzymes acting upon carbohydrates (amylase, invertase and maltase) are predominant. In *Glossina* only a very weak amylase remains, and the tryptase is the most active enzyme. In *Chrysops* there is a very active tryptase, a weak amylase and a strong invertase, though maltase is absent. In all three insects the enzymes are secreted mainly

Table I. *Distribution of digestive enzymes in* Chrysops.

Enzyme	Salivary glands	"Cardia"	"Stomach"
Amylase	–	–	±
Invertase	–	–	+ + +
Maltase	–	–	–
Lactase	–	–	–
Lipase*	?	?	?
Tryptase	–	–	+ +
Peptase	–	–	–
Peptidase	–	–	+

Table II. *Distribution of digestive enzymes in* Glossina.

Enzyme	Salivary glands	Anterior part of mid-gut	Posterior part of mid-gut
Amylase	–	–	±
Invertase	–	–	–
Maltase	–	–	–
Lactase	–	–	–
Lipase*	?	?	?
Tryptase	–	±	+ + +
Peptase	–	–	–
Peptidase	–	–	+

* Lipase was not stable in the enzyme preparations employed.

Table III. *Distribution of digestive enzymes in* Calliphora.

Enzyme	Salivary glands	Anterior part of mid-gut	Posterior part of mid-gut
Amylase	+ + +	+ +	+ +
Invertase	–	+	+ + +
Maltase	–	±	+ + +
Lactase	–	–	–
Lipase*	?	?	?
Tryptase	–	–	±
Peptase	–	–	–
Peptidase	–	–	±

* Lipase was not stable in the enzyme preparations employed.

in the posterior half of the mid-gut. The absence of enzymes from the cardia of *Chrysops* is interesting, for, as found by Cragg in *Tabanus*, and confirmed in *Chrysops*, there is a continuous secretion on the part of the cells of cardia. The nature of this secretion is unknown.

The digestive enzymes in *Chrysops* are such as were to be expected from a consideration of its diet, for only the female sucks blood. The male feeds probably on honey-dew and the nectar of flowers; and according to Austen (1909), speaking of the Tabanidae in general, "there is reason to believe that when blood is not procurable the females will also feed as the males do." In the foregoing experiments only female flies were used, and it would be interesting to know whether there is any difference in the digestive enzymes of the two sexes.

Factors affecting blood coagulation. The various parts of the alimentary tract were not tested for coagulins and anticoagulins, but it is probable that the salivary secretion prevents the coagulation of the blood, for the blood in the stomach was always in a fluid state; and Cornwall and Patton (1914) found a powerful anticoagulin in the salivary glands of *Tabanus albimedius*.

Function of the crop. In *Chrysops*, as in *Tabanus*, the newly ingested blood goes straight into the stomach; and the crop certainly does not serve as a reservoir for blood, as it does in the tsetse-fly. In the recently emerged fly the crop is distended with air, and there is no doubt that, as in the mosquito and many other insects (see Knab, 1909, 1911), one of the functions of the crop is to receive the air which the insect swallows at the time of emergence, and by means of which it keeps its abdomen distended until the body wall has hardened.

In many older flies the crop was found to contain a colourless watery fluid. Cragg (1920) observed a clear fluid in the crop of *Tabanus*, and he supposed that some of the secretion from the mid-gut had been regurgitated into it. But in *Chrysops* the clear fluid in the gut is always amber-coloured and certainly different from that in the crop. At the time of making the dissections I formed the conclusion that the fluid in the crop was saliva, and that the crop served as a reservoir for saliva, analogous to the salivary receptacle of the cockroach. But it is possible that the crop contents represent free fluid

obtained from the surface of plants, and that, as in the mosquito (Wright, 1924; Macgregor, 1930), when the insect feeds on such substances the crop does function as a temporary reservoir.

SUMMARY.

The histological changes in the gut of *Chrysops silacea* during the digestion of blood are the same as those described by Cragg in *Tabanus*.

Digestive enzymes are confined to the posterior half of the mid-gut. They comprise an active invertase, a weak amylase and an active tryptase and peptidase. These results are compared with those in *Glossina* and *Calliphora*.

The functions of the crop are discussed.

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A NOTE ON THE PRESENCE OF SUPERNUMERARY LOBES IN SALIVARY GLANDS OF ANOPHELINE MOSQUITOES

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(With Plate I.)

It is presumed that the majority of investigators who dissect anopheline mosquitoes for the purpose of detecting the parasites associated with malarial fever are not concerned with the finer details of anatomical relationships. Nor do they take the time to completely isolate the two acinus glands with the accompanying normal six lobes. This latter is assumed not essential in a search for protozoan organisms, as the sporozoites may be found in the torn portions of the acinus material as well as in the muscular tissue and mounting fluid in which the dissection is made.

One who is bent on an examination of all six lobes in hundreds or thousands of salivary glands of mosquitoes cannot help noticing an abnormality in numbers of acinus lobes when the salivary glands are removed intact. During a period covering 18 years of mosquito dissections, one of us has recorded in all 202 instances of supernumerary lobes in glands of anopheline mosquitoes. These were found amongst the following species:

Anopheles quadrimaculatus,
A. punctipennis,
A. subpictus.

The latter species yielded more than half of these abnormalities. To this list may be added *A. barbirostris*, the material having been furnished through the courtesy of Prof. C. Strickland of the Calcutta School of Tropical Medicine.

The first instance of this sort came to the notice of the senior author during the practical course in medical entomology when a student was making a dissection of the salivary glands of a specimen of *A. subpictus*. The glands, when the abnormality was recognised were observed to be still connected with the head, but were partly hidden by the neck tissues, the free end only coming to view. On removing the coverglass which held the dissected parts, the glands

were freed from the surrounding tissue and their abnormal character confirmed. The glands had eight lobes, four on each side instead of three (Pl. I, fig. 2), one lobe being forked. In order to exclude the possibility of accounting for the appearance of the parts by the superimposing of the tissue, further careful manipulations with the dissecting needles revealed the stretched lobes, whose origin was traced from the duct to the free ends.

One realises that these supernumerary forms are not noticed unless attention is directed to them. They were first recorded in the literature by Stephens and Christophers (1903)[1] who remark that, "occasionally an acinus becomes bifid at a short distance from its termination. A common abnormality also is a small accessory acinus near the proximal end of an acinus."

Prof. C. Strickland in a private communication states that, while Stephens and Christophers and the present writers comment on the comparative frequency of the condition, he has seen only two instances of abnormal salivary glands in the course of 8 years of mosquito dissections in the course of college class demonstrations. The rarity of these observations may be further exemplified by referring to the photograph (Pl. I, fig. 1) which had been used for several years in class-room demonstrations. The subject was represented to the class as the normal dissected salivary glands of a specimen of *A. quadrimaculatus*. The unusual appearance of the distended lobe was not observed except quite casually. It must be confessed furthermore that this abnormal feature escaped notice at the time when the dissection was prepared and the photograph made.

EXPLANATION OF PLATE I.

Fig. 1. The lobes of two sets of salivary glands of *A. quadrimaculatus*. One lobe (the second from the right) is shown with a distinct terminal bifurcation. (Dissection prepared and photographed by Dr Bruce Mayne.)

Fig. 2. Eight lobes of two glands dissected from an *A. subpictus*. In the photograph (to the right) may be seen one of the lobes greatly dilated and forked. Attached to this set note one pair of lobes (dark in the illustration) situated on either side of the salivary duct and appearing distinctly less refractile. On dissection, a lobe of the other set of four was noticed to have suffered considerable damage. Slight retouching of the photograph evidently restored its original appearance. (Photographed by Prof. D. N. Roy.)

Fig. 3. One set of salivary glands of a specimen of *A. barbirostris*. The mosquito showed on dissection five lobes on one side and four lobes on the other. The shortest lobes appeared to be placed between the long lobes. The normal lobes and the accessory elements were indistinguishable in character. The set of glands illustrated shows two long lobes, two shorter lobes of equal length and a still shorter irregular lobe. The other set of glands (not illustrated) possessed four lobes in pairs of unequal lengths, the shorter lying on the inside of the group in the dissection. (Photographed by Prof. D. N. Roy.)

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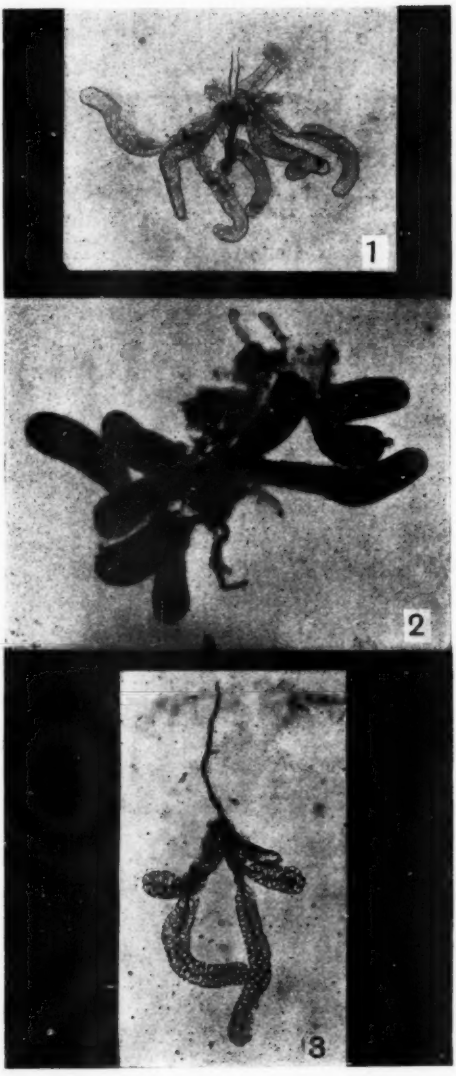
[1] Stephens, W. W. and Christophers, S. R. (1903). *The Practical Study of Malaria*, p. 133. University Press, Liverpool.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A NEW SPECIES OF THE GENUS *RAYMONDIA* FRAUENFELD (DIPTERA PUPIPARA, STREBLIDAE) WITH A NOTE ON *RAYMONDIA QUADRICEPS* JOBLING AND *R. BEDFORDI* FERRIS

By B. JOBLING.

From the Wellcome Bureau of Scientific Research, London.

(With 2 Figures.)

The description of the species is made from specimens mounted on slides, which were given to me by the late Dr J. Waterston for examination. As all the specimens were flattened and very much distorted by the pressure of the coverslips, it was impossible to determine whether they belonged to one of the known species. Permission having been received for their remounting, the following method was adopted. The slide was placed in xylol and, after the Canada balsam had dissolved, the specimen was transferred to fresh xylol and then to absolute alcohol. In this medium the wings were detached and, after being cleared in oil of cloves, mounted on a slide. The wingless body was passed through spirits of diminishing strengths to water, and then to caustic potash, and boiled. During boiling the specimens become soft and acquire their natural form. If any parts of the body are still distorted after the boiling they may be restored to their natural position with two dissecting needles. This method of remounting gave a very satisfactory result, although the specimens had been mounted more than 10 years. The further manipulation, together with the morphology of the genus, have been described elsewhere (Jobling, 1930).

Raymondia waterstoni n.sp.

Three specimens were examined, the type, a female, and two paratypes, a male and a female from a bat *Rhinolophus simulator*, from German East Africa, Iringa, Uhehe District. The type and the paratypes are in the British Museum, Natural History Department.

This species resembles *R. huberi* Frauenfeld in the structure of the head and thorax, and especially in the venation of the wings and the chaetotacy of the prescutum and the scutum, but it can be easily distinguished from the above-mentioned species by the following characters. In the female the setae of the two longitudinal rows of the dorsal surface of the abdomen are very small, immediately behind the lobes of the basal tergite-like parts; the apical

cone of the abdomen is slightly broader in its distal than in its basal part. In the male the apodemes of the genitalia are of equal length, while in *R. huberi* the apodeme of the penis is twice as long as the other apodeme. It can be distinguished from all known species of the genus by the absence of the alula.

Female (Fig. 1), 1·75 mm. long (on slide). Head quadrangular when viewed dorsally, narrower than the anterior part of the thorax (width between the tips of the humeral processes); sub-region of the vertex distinct; each laterovertex bears about six strong setae arising in the lateral and posterior parts, besides these are several very small and a few moderately strong setae along the lateral border, the middle and the anterior part of the laterovertex. On the ventral surface of the head each postgena bears moderately strong setae along the inner margin, one in the lateral part and one smaller anterior to this; the antero-lateral part of the postgena and the gena bears very small setae. Palps present no distinctive character; theca nearly as long as broad.

Thorax rounded when viewed dorsally; humeral processes moderately large, each with two strong and long and a few much smaller setae. Prescutum scantily covered with small setae, which become slightly longer in its posterior part; the mesonotal suture incomplete in the middle. Scutum with a very few small setae, two moderately strong setae in the middle. Scutellum rather narrowly rounded; two strong scutellar setae arise from the middle; the posterior margin of the scutellum bears small setae which stand at equal intervals from each other. Sternopleurae of the mesothorax and the pleurotrochantines more densely covered with small setae than the prescutum (Fig. 2 D).

Wings (Fig. 2 A), 1·75 mm. long (on slide). Humeral vein with one seta, no setae in the subcostal cell, many in the anterior third of the marginal and a few in the submarginal cells; alula absent; fifth longitudinal vein not reaching the wing margin.

Abdomen with a very distinct apical cone, which is slightly broader in its distal than in its basal part; dorsally it bears four moderately strong and long and a few small setae; the setae of the two longitudinal rows of the dorsal surface of the abdomen vary very much; immediately behind the lobes of the basal tergite-like parts they are very small, in the posterior third of the abdomen they are normally developed, very strong and long; the setae of the ventral surface of the abdomen become more or less abruptly very small and much more numerous in the antero-lateral parts; in the most posterior part of of the ventral surface of the abdomen, in front of the apical cone, the setae are stronger and arise from very large thecae (Fig. 2 B). Edge of the slightly sclerotised sternite-like part of the basal portion of the abdomen beset with stronger setae.

Male (Fig. 2 C), 1·5 mm. long (on slide), differs from female in the following characters: the setae of the two longitudinal rows of the dorsal surface of the abdomen normally developed, the apical cone of the abdomen is very much larger, slightly sclerotised and as long as broad at the base; it bears two very strong, long setae in each postero-lateral part, its dorsal surface is bare, while

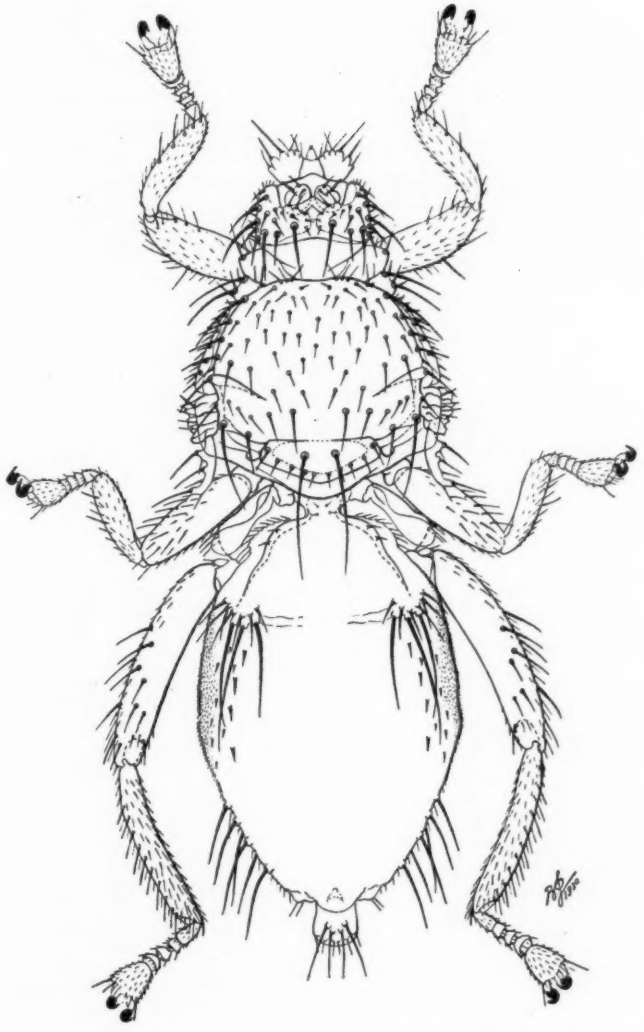

Fig. 1. *Raymondia waterstoni* n.sp. Female, wings removed.

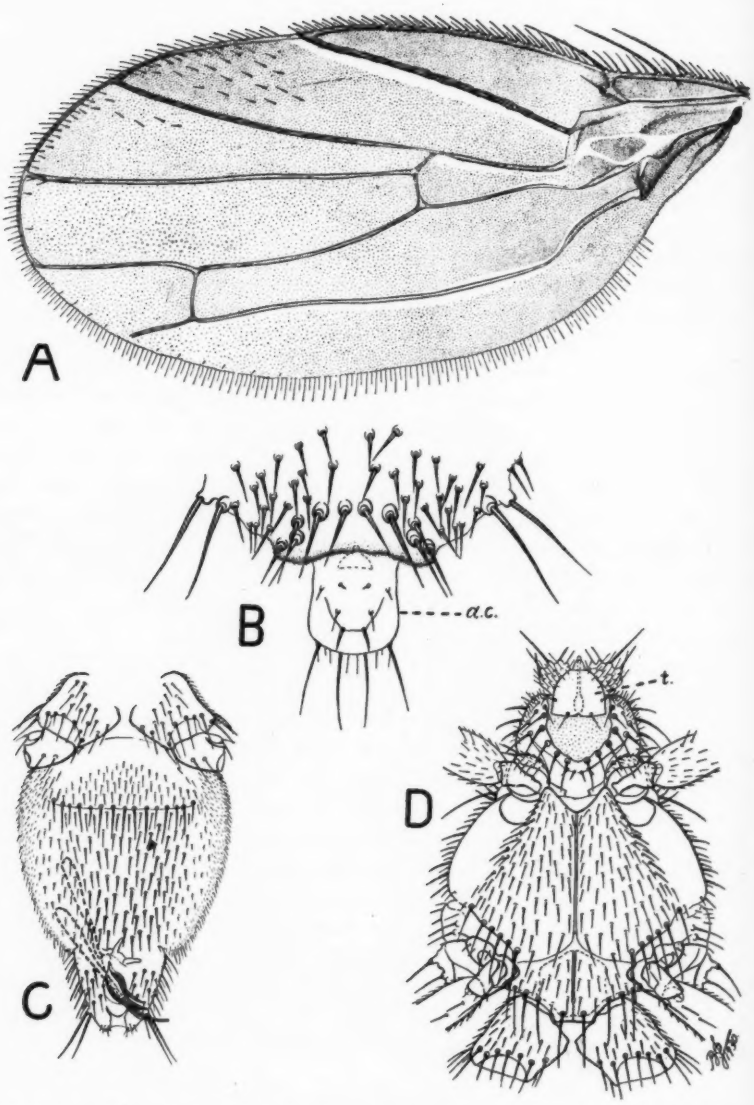

Fig. 2. *Raymondia waterstoni* n.sp. A. Wing. B. Ventral aspect of posterior end of abdomen of female; *a.c.* apical cone. C. Ventral aspect of abdomen of male. D. Ventral aspect of head and thorax of female; *t*, theca.

laterally and ventrally it has setae nearly of the same size as those arising from the middle of the ventral surface of the abdomen. Apodemes of the genitalia of equal length; they are shown on Fig. 2 C by dotted lines.

NOTE.

In a previous paper (Jobling, 1930) I described a fly to which the name *Raymondia quadriceps* n.sp. was given. In the same number of *Parasitology* there appeared a paper by Ferris (1930) describing a new species of this genus under the name *R. bedfordi*. On comparing the two flies, as described independently by Ferris and myself, it became obvious that they were identical in every respect and should, therefore, be regarded as one species. Since the manuscript of Ferris's paper was accepted for publication a few days earlier than mine and also preceded mine in the journal, the name *R. quadriceps* Jobling, 1930, becomes a synonym of *R. bedfordi* Ferris, 1930.

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A SUPPLEMENTARY DESCRIPTION OF *LYTOCESTUS INDICUS* MOGHE (SYN. *CARYOPHYLLAEUS INDICUS* MOGHE 1925. CESTODA)

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(With 6 Figures.)

Since I published (1925) a description of this interesting new species of a Caryophyllaeid, Woodland (1926) has suggested that the bodies which I described as post-ovarian vitellaria (1925, p. 233, fig. 2, *p.ov.vit.*) are in reality ovarian follicles, and that, therefore, the worm is a *Lytocestus* and not *Caryophyllaeus*. Southwell in his papers (1928 and 1930) has embodied my original description of this worm and has concluded the description by referring to Woodland's remark regarding the post-ovarian vitellaria, without definitely assigning the species to the genus *Lytocestus*. With additional material at my disposal, it was possible to re-examine its anatomy, and I have found that the bodies described and figured by me as post-ovarian vitellaria are in reality ovarian follicles. I also take this opportunity of publishing a more complete and, I hope, a more accurate description of its general anatomy.

As stated in my paper (1925), the host is the common Indian siluroid, *Clarias batrachus* Bl., and from fifteen of these fishes I recently obtained thirty more examples of this parasite. These parasites, on opening the fishes, appear as small knobs attached to the rugose wall of the stomach, and they expand in size considerably on being placed in water. Some of the parasites were flattened between glass slides and fixed in Mann's fluid; others were fixed in Zencker's fluid.

The mature unflattened parasites measure, when fixed, from 15 to 29 mm. in length and 1·82 to 2·73 mm. in maximum width, which is across the cirrus pouch and anterior to the cirrus opening. The head is short and bluntly rounded (Fig. 2 A), markedly narrower than the body and measures about 3 mm. in length and 1·2 mm. in breadth. In some species it is also marked with longitudinal furrows (Fig. 2 B, C, D) similar to those described by Woodland (1924) in *Lytocestus chalmersius* and by Fuhrmann and Baer (1925) in *Monobothriodes cunningtoni*. These furrows in some specimens commence at the extreme anterior end of the "scolex" and continue for some distance on the body, but in other specimens may commence a little posteriorly and stop at the base of the head.

The musculature is similar to that described by Woodland (1926) in *Lytocestus filiformis* (Woodland 1923), Fuhrmann and Baer (1925). The longitudinal muscle fibres are disposed in two distinct zones: a fairly thick inner or epimedullary (Figs. 4, 5, 6, *EPLM*) and an outer or cortical (*CORLM*). Further, the outer layer is internal to the nuclear layer of the subcuticula (*SUB*) and not external to it.

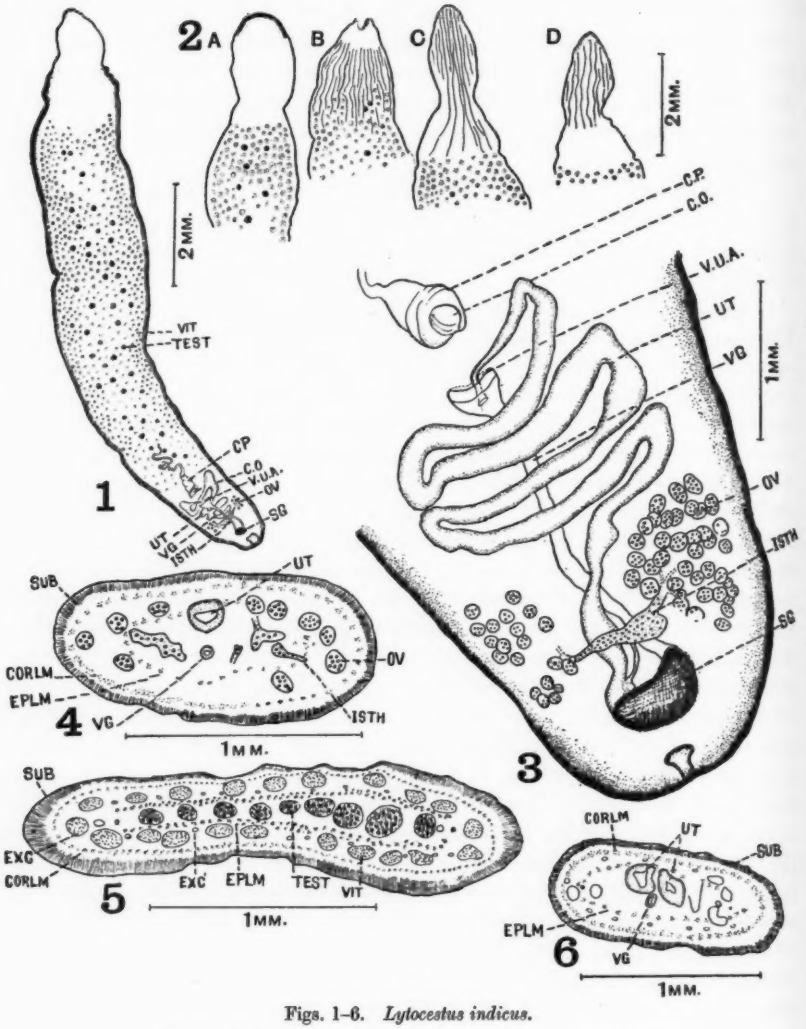
The excretory system in the testicular region (and also in the region occupied by the uterus) (Fig. 5, *EXC*) consists of two vessels, one on each side situated external to the testes, besides a number of cortical narrower vessels situated between the epimedullary and cortical muscle layers (Fig. 5, *EXC'*).

The genital apertures are situated in the last seventh of the body length. The opening of the cirrus sac (Fig. 1, *C.O.*) is quite separate from the common opening of the vagina and uterus (Figs. 1, 3, *V.U.A.*), the space between the two openings being 0·22 to 0·27 mm. The cirrus pouch (*C.P.*) is in shape not unlike a bell with a thin handle. The vagina (*VG*) is a narrow tube which pursues a slightly wavy course in the middle line; anteriorly it is dilated to receive the uterus opening, and posteriorly, behind the ovary, it runs into the shell gland and becomes continuous with the uterus. The uterus has the course shown in the figure (Fig. 3, *UT*), and does not extend anteriorly to the cirrus opening. As made out in transverse sections the walls of the uterus are thick and glandular. The most anterior loop lies between the base of the cirrus sac and the vagino-uterine aperture. The final ½ mm. of its length before joining the vagina becomes distinctly narrowed and is ciliated. The longitudinal extent of the uterus is approximately one-fifth of that of the testes.

The ovary (Figs. 1, 3, *OV*) takes the form of numerous follicles on either side and is situated anterior to the shell gland (*SG*). In none of my specimens does the posterior end of the ovarian follicles reach the anterior level of the shell gland. The follicles on either side send small ducts which, in at least three of my specimens, contained ova. There is a transverse bagpipe-shaped isthmus (Fig. 3, *ISTH*) joining the two ovarian masses. The dilated portion of the isthmus lies slightly more to one side than to the other, and hence its transverse disposition is not symmetrical. The isthmus also contains ova. From the dilated middle region of the isthmus a small duct runs backwards and joins the vagina, the union being marked by a small dilatation. As seen in transverse sections the ovarian follicles (Fig. 4, *OV*) are cortical and the isthmus (*ISTH*) is medullary.

The vitellaria, as seen in transverse sections (Fig. 5, *VIT*), lie between the cortical and medullary layers of the longitudinal muscle fibres and surround the testes. They are cortical in position. *In toto* preparations the vitellaria are seen to be more numerous in the lateral parts of the body and do not extend posterior to the vagino-uterine aperture. They measure, in surface view, from 88μ by 77μ to 112μ by 88μ, according to the size of the specimens.

The testes (Fig. 1, *TEST*) are scattered among the vitellaria, beginning a short distance behind the most anterior vitellaria and extending posteriorly



Figs. 1–6. *Lytocestus indicus.*

Fig. 1. Entire worm.
Fig. 2. Various forms of "head."
Fig. 3. Posterior extremity.
Fig. 4. Transverse section through the region of the ovary.
Fig. 5. Transverse section through the region of the testes.
Fig. 6. Transverse section through the region of the uterus.

C.O. Cirrus opening.—*CORLM.* Cortical layer of longitudinal muscles.—*C.P.* Cirrus pouch.—*EPLM.* Epimedullary layer of longitudinal muscles.—*EXC.* Main excretory vessel.—*EXC'.* Smaller excretory vessels.—*ISTH.* Isthmus.—*OV.* Ovary.—*SG.* Shell gland.—*SUB.* Nuclear layer of subcuticula.—*TEST.* Testes.—*UT.* Uterus.—*V.U.A.* Vagino-uterine aperture.—*VG.* Vagina.—*VIT.* Vitellaria.

to about the level of the junction of the cirrus sac and vas deferens. They occupy nearly two-thirds of the body length. As seen in transverse sections, they lie (Fig. 5, *TEST*) internal to the epimedullary layer of the longitudinal muscle fibres and are disposed in a single layer. They measure on an average 119μ by 95μ.

The eggs lie in the convolutions of the uterus and measure (in balsam) approximately 80μ by 40μ.

It will be evident from the foregoing description that the worm belongs to the genus *Lytocestus* Cohn 1908, as emended by Woodland (1926). The longitudinal muscles consist of two layers, as in *Caryophyllaeus*, but the cortical layer is internal to the nuclear layer of the subcuticula and not external to it as in the latter genus. Further, the vitellaria are cortical and are wholly absent behind the ovary. The follicles of the ovary are cortical and the isthmus medullary.

The following are the specific characters of the new species: Body broad and flat, 15–29 mm. in length and from 1·82 to 2·73 in maximum width. Longitudinal musculature consists of two layers (cortical and epimedullary) both lying internal to the nuclear layer of the subcuticula. The longitudinal extent of the uterus is approximately one-fifth that of the testes. Male and female genital pores separate; uterine and vaginal pores common. Vitellaria are cortical and do not extend beyond the vagino-uterine aperture, and measure 77μ to 88μ by 88μ to 112μ. Testes measure on an average 119μ by 95μ. Ovarian follicles are cortical and the isthmus is medullary; ovary does not extend beyond the shell gland. Vagina, a median tube, which is joined by a small duct from the isthmus, joining the uterus in the shell gland. Uterus a much convoluted tube, not extending anterior to the cirrus opening. Eggs (in balsam) measure approximately 88μ by 40μ. Parasite in the intestine of the siluroid fish, *Clarias batrachus* Bl. Locality: Central Provinces, India.

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SOME FRESH-WATER LARVAL TREMATODES FROM CHESHIRE

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(With Plates II and III including 15 Figures.)

CONTENTS.

	PAGE
I. Introduction	88
II. Echinostome cercariae	89
(1) *Cercaria oscillatoria* n.sp. and cyst	89
(2) *Cercaria limbifera* Seifert, cyst and excysted larva	91
III. Fork-tailed cercariae	94
(1) *Cercaria echinomorpha* n.sp.	94
(2) *Cercaria chromatophora* n.sp.	95
(3) *Cercaria pygocytophora* n.sp.	96
References	97
Explanation of Plates	98

I. Introduction.

The larval trematodes described in this paper were obtained from fresh-water molluscs collected from Rostherne Mere and a pond near Ashley, both in Cheshire. Rostherne Mere is a large sheet of water and carries an extensive fauna, vertebrate and invertebrate. The molluscs were collected from various fixed positions in the littoral region, and this method has confirmed previous observations (unpublished) in other stretches of fresh-water on the variation in the distribution and localisation of infected molluscs. How far this may be related to movements of the final host or to physical factors affecting the migration of the molluscs is at present uncertain; further observations are being made in an attempt to elucidate this problem. The molluscs were isolated in the laboratory in 3×1 glass specimen tubes, when the infected specimens were easily seen by the swarming of the cercariae, and all observations (including measurements) were made on these living, fully developed cercariae. Infected snails were later crushed in physiologically normal saline in order to obtain living sporocysts or rediae for examination. Measurements are, of necessity, only approximate owing to the slight pressure exerted by the weight of the cover-glass and the great powers of contraction and extension of these larval forms. Neutral red was used as an *intra vitam* stain; it also inhibits movement. Of the five species described four are new, the fifth, for which a

further stage in the life history is described, being little known and not previously recorded for this country.

II. Echinostome cercariae.

(1) **Cercaria oscillatoria** n.sp. and cyst.

(Pl. II, figs. 1–4.)

C. oscillatoria develops in elongate orange-coloured rediae, capable of independent movement, in the digestive gland of *Planorbis carinatus*. The young rediae, which are almost transparent, contain at this period of the year (July) germ balls of varying size, but it is impossible to state whether these will give rise to daughter rediae or to cercariae. These young forms differ from the older ones containing developing and mature cercariae in that the former possess a well-developed collar and birth pore together with pronounced ambulatory processes. Some of the older rediae, though possessing a well-marked pharynx and long intestine (with dark brown or black contents) extending posteriorly for about two-thirds of the body length, have the general appearance of a sporocyst. The collar, though present, is not so well marked, and the ambulatory processes project little from the body. The rediae naturally vary in size from the youngest form obtained, 0·42 mm. long and 0·12 mm. wide, up to the mature form, 1·64 mm. long and 0·25 mm. wide. All rediae possess a well-defined excretory system. In the young stages it is possible to count the individual flame cells, but in later stages they are extremely numerous, very close together and difficult to count. The flame cells are comparatively large and very active. The bilaterally symmetrical excretory system comprises, on each side, two groups of flame cells, one running into a very short anterior and the other into a long posterior collecting duct, the ducts discharging into a large excretory vesicle, situated nearer the anterior than the posterior end of the body. The excretory vesicles open to the exterior by small pores. In the youngest redia obtained (0·42 mm. × 0·12 mm.) the two anterior groups of flame cells lie slightly posterior to the collar, approximately midway between the pharynx and ambulatory processes, and consist of six flame cells each, their fine ducts radially arranged and joining to form extremely short collecting ducts which immediately open into the excretory vesicles. The two posterior groups of flame cells, also six in number and similarly disposed, lie at the bases of the two ambulatory processes. The fine ducts from each group lead into long posterior collecting ducts, which run forwards and discharge into their respective excretory vesicles, close to the anterior collecting ducts. In fully developed rediae flame cells to the number of fifty were counted in one group; there were many more. It was impossible to ascertain whether the number in each of the four groups was the same. The cercaria which emerges from the birth pore of the redia is almost transparent and possesses an extremely long tail. The body (0·21 mm. long), which conforms in shape to the echinostome type, is very muscular and capable of considerable contraction, so that when the

cercaria is swimming the body is almost spherical. Swimming is performed by vigorous **S**-shaped movements of the extremely long tail (1·8 mm.), but occasionally the cercaria falls through the water in an oscillating manner. The oral sucker is apposed approximately to the tip of the tail, so that the complete larva forms a slightly open ring; movement then proceeds in waves, in a plane at right angles to the ring, starting at the anterior end of the body and passing through the tail so that the cercaria oscillates in the water and gradually falls.

When extended the body assumes the typical echinostome shape, the anterior end bearing a distinct collar carrying a single row of spines. The collar is continuous on the dorsal surface, but on the ventral surface the edges turn inwards towards the junction of the pharynx and prepharynx where they end. The spines are but feebly developed; it is impossible to count them, but there appear to be two groups of five "angle" spines, one on each of the ventro-lateral lappets of the collar. Both dorsal and "angle" spines appear to be of the same size.

The well-developed oral sucker measures 0·044 mm. × 0·051 mm., whilst the larger heart-shaped ventral sucker, situated behind the middle of the body, measures 0·051 mm. × 0·055 mm. The prepharynx is short, pharynx well developed (0·017 mm. diameter), oesophagus long and bifurcating just anterior to the ventral sucker into two intestinal caeca extending to the posterior end of the body. In most echinostome cercariae the alimentary canal consists of an apparently single row of finely granular cells, but in this form no cell outlines can be seen, the canal has a smaller diameter and is full of very fine granules. Two patches of yellow pigment granules lie one on each side of the prepharynx just behind the posterior edge of the oral sucker. In some larvae other patches occur in regions along the oesophagus and near the anterior edge of the ventral sucker. The cystogenous cells, which lie under the cuticle over the whole body except in the anterior region immediately behind the oral sucker and a small area behind the ventral sucker, are large and conspicuous, the cells being filled with long slender rod-like bodies. When the cercaria is much contracted, the ventral surface of the body shows two very clear hemispherical areas, one anterior and one posterior to the ventral sucker. The cuticle appears to be pitted with small ⊥-shaped depressions, the function of which is unknown.

The excretory system conforms to the echinostome type and consists, on either side of the body, of a duct which arises from three flame cells situated near the oral sucker, and which runs to the posterior end of the body where it loops back upon itself and passes forward to the level of the pharynx. Here it again forms a narrow loop, widens considerably, and passes posteriorly as the main excretory canal, turning inwards at a sharp angle dorsal to the ventral sucker and then passing straight back to join the duct from the other side, and to form a single short narrow duct which discharges into a large spherical or pear-shaped contractile excretory vesicle. When the excretory vesicle is contracting rhythmically, a transversely elongated dilatation appears on the

short single duct in between the main canals and the excretory vesicle. The excretory pore is well defined and is sub-terminal. There does not appear to be a duct running from the excretory vesicle into the tail, as is the case in most echinostome cercariae.

The most conspicuous part of the excretory system is the two main collecting canals in the region of the body anterior to the ventral sucker. These canals are very wide and full of clear spherical calcareous concretions which appear to be formed of concentric layers. In most cases two spherical concretions have grown together and in several instances even three. In other echinostome cercariae which I have examined the concretions are single and separate spheres.

The genital system is represented by two masses of small round cells, one just behind and the other near the anterior edge of the ventral sucker.

The cyst.

(Pl. III, fig. 15.)

Several cysts were obtained from the crushed bodies of infected *P. carinatus*. These appear to be the encysted stage of *C. oscillatoria*, though the only character which suggests the relationship is the compound nature of the calcareous concretions in the anterior part of the main collecting canals. The cyst is spherical, 0·154 mm. in diameter, the double wall being 0·009 mm. thick, the rolled-up cercaria completely filling the cavity. It was impossible to remove the worm from the cyst for further observations.

(2) **Cercaria limbifera** Seifert, cyst and excysted larva.

(Pl. II, fig. 5.)

C. limbifera was discovered near Breslau and briefly described by Seifert (1926). I can confirm most of his observations, and add several important morphological features. My measurements are slightly less than those of Seifert, but no significance can be attached to this, since he apparently made his measurements, in most cases, when the cercaria was fully extended.

The cercaria develops in rediae in the digestive gland of *Limnaea palustris*. The rediae are yellowish orange in colour, and vary in size up to 2·2 mm. in length. In most stages the collar and ambulatory processes are well developed. A terminal muscular pharynx leads into a sac-like intestine which extends as far back as the ambulatory processes. On the body wall surrounding the anterior end of the pharynx is a number of so-called sensory hairs, first discovered and described for other trematode larvae by Ssinitzin (1904). Fine hairs project from the surface, and appear to have their origin in small, clear round cells in the body wall. I have not previously noticed these cells in other echinostome rediae, and they are not present in the redia of *C. oscillatoria*. The redia possesses a well-defined excretory system similar to that described for the redia of *C. oscillatoria*; the number of flame cells increases with the

growth of the redia. In the smallest redia (0·11 mm. long) there were only three flame cells in each group; in a redia 1·37 mm. long there were forty-seven cells in one group alone.

The comparatively large cercaria is 0·87 mm. long and 0·41 mm. wide; the tail, which is longer than the body, is 0·99 mm. long, and bears for the posterior two-thirds of its length a well-marked dorso-ventral fin fold. On the tail itself are numerous closely set and well-defined transverse striations. At the anterior end of the body is a collar carrying a double row of well-developed spines, thirty-five in number (thirty-seven, Seifert, *loc. cit.*) and equal in size. Ten of these spines are arranged in two groups of five each, the so-called "angle" spines, one group on each of the ventro-lateral lappets of the collar. Eight "sensory" fine hairs, similar to those in the redia, occur on the extreme tip of the anterior end of the body.

The oral sucker is 0·088 mm. in diameter, and is smaller than the ventral sucker (0·16 mm. × 0·15 mm.) which lies behind the middle of the body. The short prepharynx is followed by an elongate oval pharynx (0·05 mm. × 0·04 mm.) and a long oesophagus, bifurcating anterior to the ventral sucker into two diverticula which extend to the posterior end of the body. The oesophagus and diverticula consist of a single row of large granular cells. On each side of the oesophagus extending as far back as the bifurcation are numerous small gland cells, the ducts from which may be clearly seen near the prepharynx and on the dorsal side of the oral sucker. Their function is unknown. Cystogenous gland cells lie under the cuticle over the greater part of the body.

The excretory system conforms to the echinostome type, and is similar to that of *C. oscillatoria*. The calcareous excretory granules, however, are considerably smaller and are single spheres. Seifert (*loc. cit.*) was unable to trace out the posterior part of the excretory system, but it is present and similar to that of *C. oscillatoria*. The return duct from the posterior loop is ciliated in small patches along its length as far forward as the middle of the oesophagus. These ciliated patches appear to be broad flame cells developed in the wall of the duct. The excretory vesicle and pore are conspicuous; a fine duct runs from the vesicle into the tail and soon bifurcates into two fine ducts which end on the edges of the tail. Numerous flame cells join the first descending limb of the lateral ducts by means of very fine capillaries which can be easily seen from the ventral surface of the body.

The reproductive system is represented by four masses of small round cells, three posterior to, and one anterior to the ventral sucker. Of the three groups behind the ventral sucker the anterior is the rudimentary ovary and the two posterior groups the rudiments of the testes.

The cyst.

(Pl. III, fig. 7.)

The cercaria encysts in the body, outside the proximal part of the alimentary canal of *L. palustris*. The cyst wall is very thick, and appears to be composed of concentric layers, and two definite walls can be distinguished. The spherical cysts have a diameter of 0·385 mm. and the complete wall is 0·033 mm. thick. The cercaria lies curled up within the cyst; the suckers, excretory granules and spines are fairly conspicuous.

Excysted immature trematode.

(Pl. III, figs. 8, 9.)

The young worm when removed from the cyst is a typical echinostome, and shows a considerable advance in development from the cercaria stage. The body is 1·092 mm. long and 0·23 mm. wide in the anterior region, but it tapers slightly behind the ventral sucker. The collar is well marked and carries thirty-five equal spines, 0·026 mm. long and 0·0057 mm. broad, disposed as in the cercaria. The oral sucker has a diameter of 0·09 mm., and the ventral sucker now situated well in front of the middle of the body measures 0·194 mm. × 0·18 mm. The anterior region of the body as far back as the ventral sucker carries numerous, equally spaced, transverse rows of small spines. The alimentary canal has lost its cellular appearance, but the disposition of its parts is similar to that of the cercaria. The gland cells round the oesophagus, together with their ducts, show no further development. The excretory and genital systems show a marked development. In the former the collecting ducts have increased in diameter, and the ciliation of the ascending limb is a distinct and conspicuous feature of the excretory system. In the region of the body, anterior to the ventral sucker, the main collecting canal, even into the loop, has developed distinct lateral branches, a feature of adult echinostomes. The excretory vesicle is bulbous and the pore sub-terminal. The oval testes lie one behind the other in the middle line, approximately midway between the ventral sucker and the posterior end of the body. The ovary is anterior to them, and, although the vitellaria are still undeveloped, the vitelline ducts can be distinguished. The uterus runs forward between the branches of the main collecting canals of the excretory system towards the ventral sucker, beyond which it cannot be traced. The cirrus sac, which lies by the side of, and with its opening anterior to, the ventral sucker, is clearly defined. It extends posteriorly for approximately half the length of the ventral sucker. As further growth in length of the worm may alter the relative positions and size of the various organs, specific determination is impossible, and suggestions with regard to its final host are purely speculative. In all probability the worm is a new species.

III. Fork-tailed cercariae.

The following three new species of fork-tailed cercariae fall into two different groups, one, *C. echinomorpha* n.sp., is an apharyngeal form, whilst the other two, *C. chromatophora* n.sp. and *C. pygocytophora* n.sp., are pharyngeal forms which, in all probability, enter a second intermediate host and develop into holostome larvae.

(1) **Cercaria echinomorpha** n.sp.

(Pl. III, figs. 10, 11.)

Miller (1926, 1927) has attempted to classify all existing fork-tailed cercariae, and has brought together into one group, the "Elvae" group, those large apharyngeal longi-furcate forms with several morphological features in common, and which appear to be related. The only two European forms which have been described so far are *C. ocellata* de la Val. (Ssinitzin, 1909) and *C. macrosoma* (Brown, 1926). *C. echinomorpha* n.sp. conforms to most of the definitive characters of the above group and must be added to it.

This cercaria develops in slender, white, elongate sporocysts, which form a tangled mass in the digestive gland of *Planorbis carinatus*. The sporocysts are constricted at intervals and, between the constrictions, bulbous swellings, containing developing cercariae, appear. *C. echinomorpha* swims actively by means of the forked tail, but appears to be able to progress equally well either body or tail foremost. At times the cercariae fix themselves (to the sides of the glass vessel containing them) by means of the ventral sucker, about which they pivot through 180° by making vigorous **S**-shaped lateral movements of the tail. These movements take place usually near the surface of the water and on the side of the vessel nearest to the source of light. At other times the cercariae may float passively on the surface of the water when the comparative heaviness of the translucent body contrasts with the delicacy of the long slender tail.

The oval body of the cercaria is 0·25 mm. long and 0·105 mm. wide, and the posterior part tapers towards the base of the tail. The latter, including the forks, is approximately twice as long as the body and is capable of considerable extension. The measurements are: tail stem, 0·35 mm.; forks, 0·12 mm. The tail also tapers from 0·035 mm. at the base to 0·026 mm. at the bifurcation, the forks being somewhat constricted at their junction with the tail stem. In addition the forks bear a narrow dorso-ventral fin fold, approximately half the width of the middle portion of the fork. Both the body and the tail (including the forks) are spined, and on the body the transverse rows are more closely set in the region of the oral sucker. The most conspicuous feature of the cercaria is the eye-spots, two in number, and which consist of a cup formed of black pigment granules surrounding a definite lens. The eye-spots are 0·009 mm. in diameter.

The pear-shaped oral sucker (with the large end directed anteriorly),

0·08 mm. long and 0·06 mm. wide, is approximately a third of the body length, and the ventral sucker, which varies in shape and is situated behind the middle of the body, is 0·04 mm. wide and 0·03 mm. long. Muscle bands, four in number, stretch between the dorsal body wall and the sides of the sucker. The actual mouth opening could not be seen, but continuous with the narrow posterior end of the oral sucker is a very narrow sinuous oesophagus, which bifurcates anterior to the ventral sucker into two diverticula which appear to end just by it. A so-called "head-gland" is contained within the oral sucker and is composed of numerous spherical cells. The "penetration" (Miller, 1926) gland cells (usually designated salivary gland cells) together with their ducts are very conspicuous, but it is difficult to make out their number in the living animal. There appear to be five pairs, possibly six, two pairs anterior to and three pairs posterior to the ventral sucker, and completely filling the posterior part of the body. In a related form, *C. ocellata*, and also in some other members of the "Elvae" group, these gland cells are differentiated into two sets, an anterior set with coarsely granular cytoplasm, and a posterior set with homogeneous cytoplasm. The gland cells of *C. echinomorpha* appear to be of one type only with granular cytoplasm. The ducts arising from the cells are heavy and sinuous and open on the tip of the oral sucker.

The bilaterally symmetrical excretory system comprises anterior and posterior lateral ducts which unite near the ventral sucker to form the main collecting ducts, somewhat convoluted at first, but eventually tracing an almost straight course to the posterior end of the body, where they discharge into the excretory vesicle. The main collecting ducts are ciliated internally in the convoluted region only. From the excretory vesicle a fine duct passes down the tail stem. There are two sets of six flame cells in the body, and two cells in the proximal part of the tail stem; each anterior and posterior lateral duct receives three, but the capillaries from the flame cells to the posterior lateral duct cannot be distinguished. Altogether there are fourteen flame cells, twelve in the body and two in the tail. Further history unknown.

(2) **Cercaria chromatophora** n.sp.

(Pl. III, fig. 12.)

This cercaria, which develops in elongate white sporocysts in the digestive gland of *Limnaea stagnalis*, can be recognised by the presence of small areas of pale yellow pigment granules in certain regions of the body. The aggregations of pigment granules vary slightly in extent, and may be loosely scattered over the anterior half of the body, though in the majority of cercariae they occur near the anterior edge of the intestinal caeca near their junction with the oesophagus, and also between the ventral sucker and the intestine fork. The cells forming the central core of the tail stem also contain yellow pigment granules, but on account of their distribution they are not as conspicuous as those in the body.

The cercaria is extremely active and capable of considerable extension. The oval body is 0·27 mm. long and 0·05 mm. wide. The complete tail is nearly twice as long as the body, the tail stem being 0·23 mm. long, whilst the forks are 0·25 mm. in length. The tail stem is furnished with long fine hairs; seven could be distinguished on each side of the stem near the forks.

The body is spined as far back as the ventral sucker, the most anterior transverse rows being more closely set in the region of the oral sucker. This protrusible sucker is well defined, and when the cercaria is somewhat extended is oval in shape, 0·06 mm. long and 0·03 mm. wide. The opening of the oral sucker leads into a short vestibule or prepharynx, followed by a muscular pharynx leading into a relatively short oesophagus which bifurcates into two diverticula, the lumen of each limb increasing in diameter as it passes posteriorly and terminates at the posterior end of the body.

The ventral sucker, behind the middle of the body, has a diameter of 0·03 mm.; its inner free edge is beset with two to three rows of fine spines. Behind the ventral sucker are four gland cells, the first two situated one behind the other, and the remaining pair side by side behind them. The ducts from these cells are most conspicuous in the region of the oral sucker, where they are full of tiny granules and open on the extreme tip of that organ.

The excretory system consists of paired anterior and posterior lateral ducts, uniting on each side to form the main collecting ducts, which discharge into a bilobed excretory vesicle at the posterior end of the body. A fine duct passes from the excretory vesicle down the central core of the tail stem and bifurcates at the fork, so that each duct passes into the tail forks. The main collecting ducts are convoluted and ciliated internally for the first part of their course. Each anterior lateral duct receives capillaries from three flame cells in the anterior region of the body, and each posterior lateral duct which arises from two flame cells in the tail stem receives capillaries from four flame cells in the posterior part of the body. Altogether there are eighteen flame cells, fourteen in the body and four in the tail.

The reproductive system consists of a mass of small round cells anterior to the excretory vesicle.

Further history unknown.

(3) **Cercaria pygocytophora** n.sp.

(Pl. III, figs. 13, 14.)

C. pygocytophora develops in very slender elongate motile sporocysts in the digestive gland of *P. carinatus*. The sporocysts are irregular in shape, being alternately swollen and constricted; they are difficult to isolate, but incomplete portions 2–3·5 mm. long were obtained. The swollen areas are full of developing cercariae. This larval form which is negatively phototropic is comparatively small. The oval body is 0·13 mm. long and 0·06 mm. wide, whilst the tail stem, 0·14 mm., is longer than the body, and the forks, 0·15 mm.,

are slightly longer than the tail stem. This part contains eight pairs of oval cells, the last pair of which often project slightly, one into each fork. The presence of these cells in the tail has been noticed by Miller (1926) and Cort (1928) and is reminiscent of *C. micromorpha* Brown (1926). At the sides of the tail stem are other small clear oval cells, superficially similar to nematocysts. The function of both these types of cell is unknown. Four long fine hairs are present on each side of the tail stem.

The anterior part of the body and the free edge of the ventral sucker are spined. The well-defined oral sucker is 0·035 mm. long and 0·026 mm. wide, the diameter of the ventral sucker being 0·03 mm. A pharynx, oesophagus and intestinal caeca are present. The eight pairs of finely granular gland cells lie in pairs, one behind the other, in the posterior region of the body. The ducts are clearly defined and run forward to open on either side of the mouth opening.

The main outline of the excretory system is similar to that of *C. chromatophora*, with the following important differences. There is only one pair of flame cells in the tail, and there are only two flame cells discharging into each anterior lateral duct. The total number of flame cells is fourteen (seven on each side of the body, including the tail). In addition there does not appear to be any ciliation in the convoluted portion of the main collecting ducts.

An important additional feature in *C. pygocytophora* is that, at the junction of the lateral ducts with the main collecting duct, another duct arises and runs forward on each side anterior to the ventral sucker. Before reaching the middle line of the body the two ducts end blindly. It would appear that these two ducts are the beginnings of the transverse duct, which is a feature of the next larval stage in the development of a holostome.

The genital system is represented by a small group of cells anterior to the excretory vesicle.

Further history unknown.

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EXPLANATION OF PLATES II AND III

PLATE II

Fig. 1. *C. oscillatoria.* Length: body 0·21 mm.; tail 1·8 mm.
Fig. 2. *C. oscillatoria.* Anatomy—calcareous concretions omitted from right main collecting duct.
Fig. 3. Young redia. Length 0·42 mm.—excretory system of one side only.
Fig. 4. Redia. Length 1·64 mm.
Fig. 5. *C. limbifera.* Anatomy: length 0·87 mm.
Fig. 6. Redia of *C. limbifera.* Length 1·32 mm.

PLATE III

Fig. 7. Cyst of *C. limbifera.* Diameter 0·385 mm.
Fig. 8. Excysted immature trematode—posterior excretory system omitted from right side of body. Length 1·09 mm.
Fig. 9. Head of excysted trematode—arrangement of spines.
Fig. 10. *C. echinomorpha.* Length 0·25 mm.
Fig. 11. *C. echinomorpha.* Anatomy.
Fig. 12. *C. chromatophora.* Anatomy, length 0·27 mm.
Fig. 13. *C. pygocytophora.* Length 0·13 mm.
Fig. 14. *C. pygocytophora.* Excretory system.
Fig. 15. Cyst of *C. oscillatoria*?. Diameter 0·154 mm.

NOTE. In cercariae, measurements refer to body only.

KEY TO LETTERING OF PLATES

a.l.d. anterior lateral duct; *a.l.e.* ascending excretory limb; *b.p.* birth pore; *c.c.* calcareous concretions; *d.l.e.* descending excretory limb; *e.p.* excretory pore; *e.v.* excretory vesicle; *f.c.* flame cell; *g.p.* genital pore; *g.s.* genital system; *l.* lens; *o.* ovary; *p.c.* pigment cup; *p.g.* penetration gland cells; *ph.* pharynx; *p.l.d.* posterior lateral duct; *s.h.* sensory hairs; *t.* testes; *t.d.* transverse duct; *v.* vitelline duct; *y.p.* yellow pigment granules.

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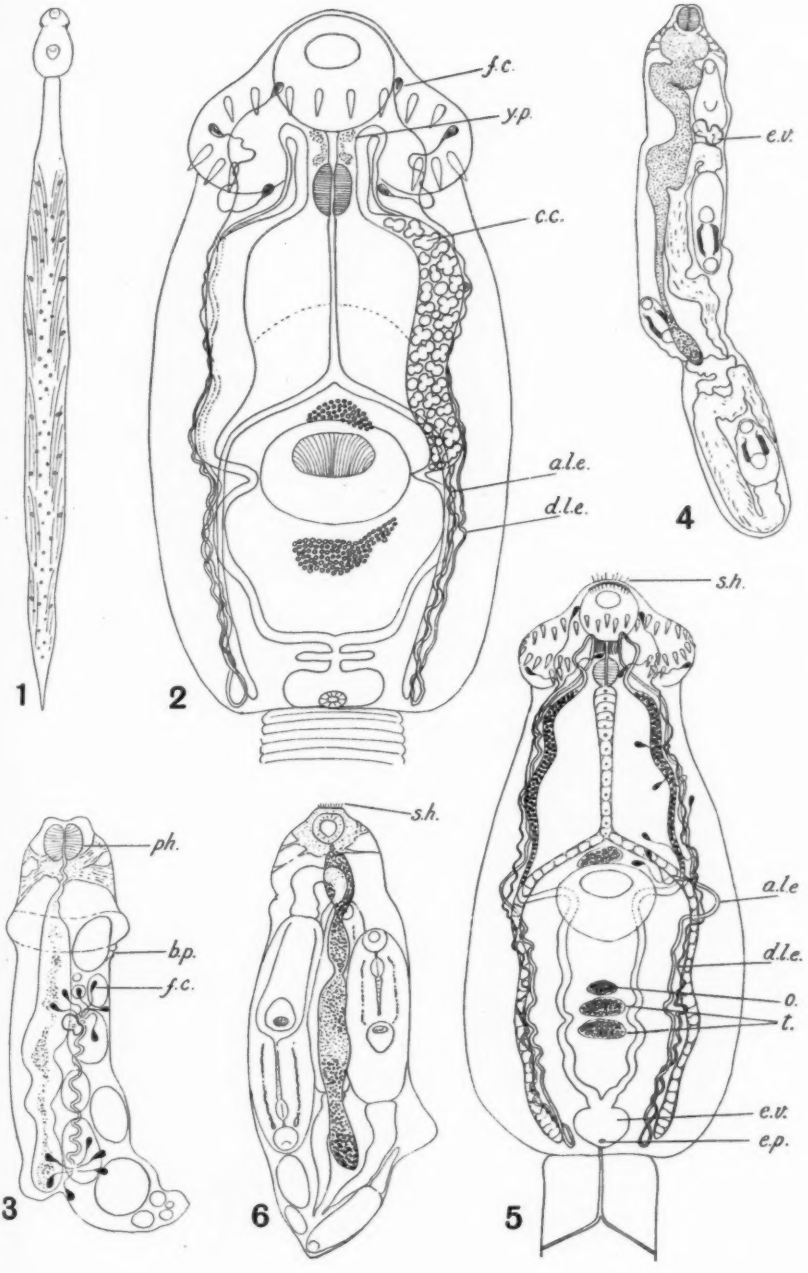

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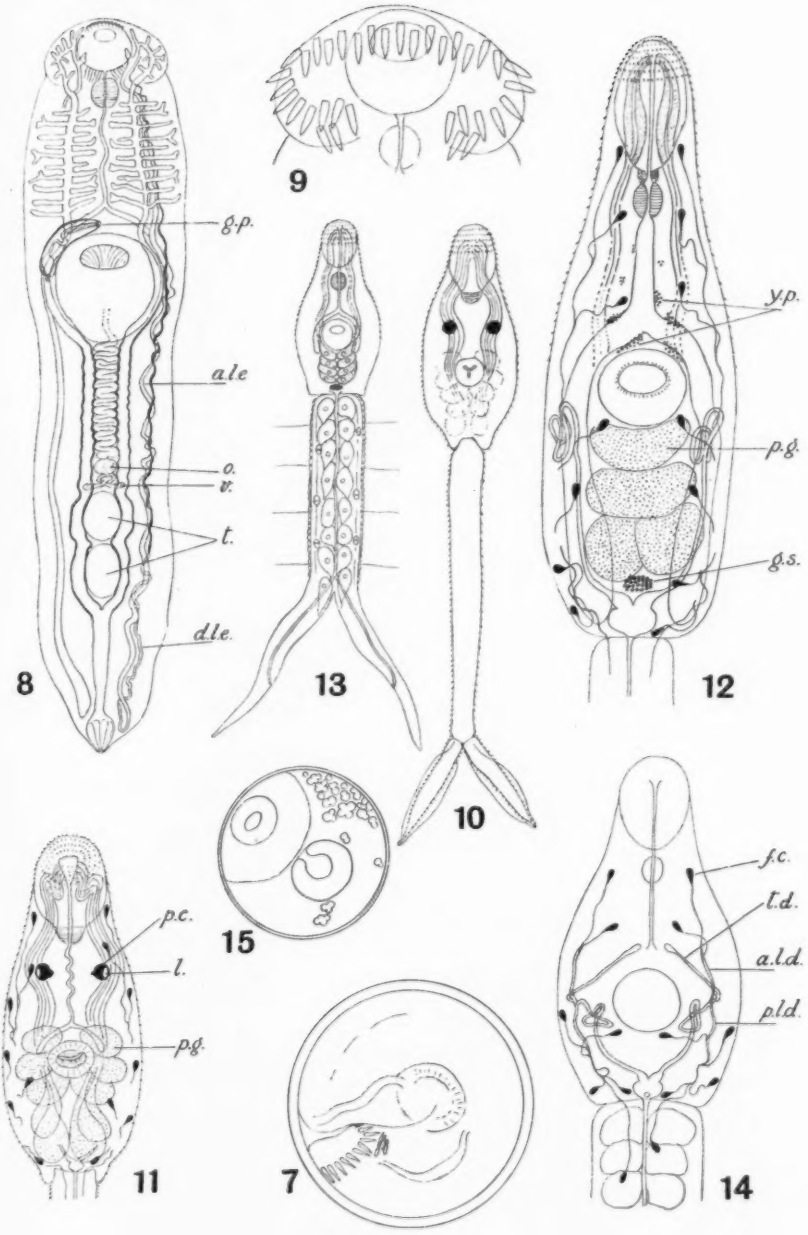

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TWO NEW TREMATODES FROM REPTILES: *PARYPHOSTOMUM INDICUM* N.SP. AND *STUNKARDIA DILYMPHOSA* N.G., N.SP.

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(With 5 Text-figures.)

Introduction.

I am indebted to my friends, Messrs M. A. Moghe, of the College of Science, Nagpur, and W. V. Nene, Officer-in-charge of the Time-Ball Observatory, Rangoon, for the material described in this paper. The trematodes were obtained by the former from the duodenum of *Uromastix hardwickii* (the spiny-tailed lizard) brought to Nagpur from Northern India, and Mr Nene gave me the tortoise, *Batagur baska*, which was found to be heavily parasitised with nematodes, along with two flukes. In both instances the trematodes proved new to science.

Paryphostomum indicum n.sp.

About two dozen specimens of this species were found in the duodenum of *U. hardwickii*. The worms are elongated, somewhat elliptical in shape, measuring 7·5–8·1 by 1·7–2 mm., the maximum breadth being in the region of the testes. The cuticle is beset with numerous strong spines anterior to the ventral sucker; posteriorly the spines become sparser and disappear completely at the level of the ovary.

At the anterior end is an oral sucker, which is longer antero-posteriorly than laterally, measuring 0·18 mm. in transverse diameter. The prepharynx follows the oral sucker. Both the oral sucker and prepharynx are surrounded by a dorsally unbroken and ventrally open kidney-shaped collar. It is beset with 42 spines disposed in two rows, out of which 32 are marginal and 5 on each side are terminal. The spines of both the rows are similar in size; the smallest measures 0·043 by 0·015 mm. and the largest 0·08 by 0·02 mm. The pharynx is a globular, muscular body, measuring 0·17 mm. in diameter. It is followed by a short oesophagus, 0·24 mm. long, which bifurcates into two simple intestinal caeca, passing laterally behind, almost to the posterior end of the body. The ventral sucker is large, having a funnel-like prolongation posteriorly, measuring 0·62 mm. in diameter, and is situated centrally at about one-fifth the length of the body from the anterior end. The ratio between the two suckers is 2 : 7.

The excretory pore is situated at the posterior end of the body, which leads into a Y-shaped bladder. The stem of the latter is long and passes anteriorly as far as the posterior testis, where it branches into two long arms passing anteriorly along the sides of the body, internal to the intestinal caeca.

The nervous system consists of two transverse, stout bands lying on the dorsal side of the pharynx. The anterior one gives off two short nerves which supply the anterior part of the body. The posterior one also gives off two long nerves which pass backwards postero-laterally. From these nerves, as well as from the lateral borders of the bands, smaller nerves emerge.

The testes are large, situated centrally in the intracaecal area, and occupy slightly more than one-third the space of the body. They are branched: the branches varying from 3 to 7; some of the branches of both the testes divide again into two sub-branches. In some cases one of the branches of the testis is more swollen than the others and appears sac-like. In sections the appearance of this swollen branch appears to be similar to the other branches, excepting that the germ cells are more closely situated. The anterior one measures approximately 1·17 by 0·92 mm. and the posterior one 1·43 by 0·88 mm. From the centre of each testis is given out a fine, long vas efferens, which passes anteriorly along the inner side of the vitellaria and turns towards the centre of the body, posterior to the hinder end of the cirrus sac, where it meets with its fellow and forms a short duct, the vas deferens, which enters the cirrus sac. The cirrus sac is situated on the dorsal side of the ventral sucker and extends from its anterior border to about 0·4 mm. behind it. It is long, and contains at its posterior end a large vesicula seminalis followed by a pars prostatica of moderate size and a strong muscular penis. The genital pore is situated immediately anterior to the ventral sucker.

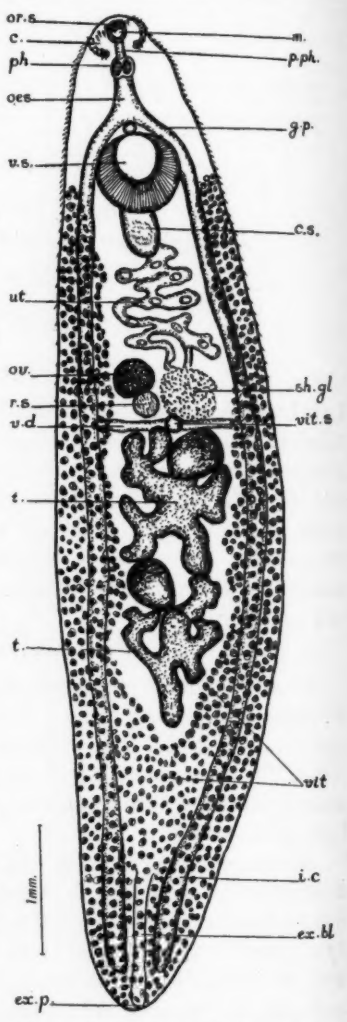

Fig. 1. *Paryphostomum indicum* n.sp. Ventral view.

The ovary is an oval body lying on the right side of the central line, anterior

to the testes, and measuring about 0·29 by 0·24 mm. Receptaculum seminis is situated posterior to the ovary and measures 0·5 by 0·38 mm. The shell gland lies centrally, immediately in front of the anterior testis. The uterus is not very long, and consists of a few coils lying between the shell gland and the posterior end of the cirrus sac. The vagina passes dorsally to the ventral sucker, and opens to the exterior by the genital aperture. The uterus contains very few eggs, which are operculated and measure 0·041–0·097 mm. by 0·033–0·072 mm. The vitellaria consist of many rounded follicles, each containing about 10 acini, lying along the dorsal side of the body and extending from the centre or the posterior border of the ventral sucker to the posterior end of the body. The glands of either side meet with each other in the central line immediately behind the posterior testis. Two short, transverse vitelline ducts pass centrally immediately in front of the anterior testis, and meet in the middle line to form a yolk sac, from which a short duct leads to the octype surrounded by the shell gland.

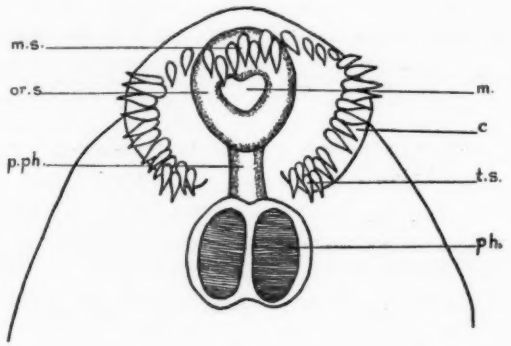

Fig. 2. *Paryphostomum indicum* n.sp. Head collar showing arrangements of spines.

The present form, having a kidney-shaped collar beset with a double row of spines surrounding the oral sucker, is contained in the family Echinostomidae Looss, 1902. Among the large number of genera of this family it comes much nearer to the genus *Paryphostomum* Dietz, 1909; the points of agreement being (1) double row of dorsally unbroken spines of equal size, (2) ventral sucker having a posterior funnel-like prolongation, (3) cuticle being anteriorly covered with thickly situated spines, (4) ovary being situated on the right side of the body, (5) vitelline follicles meeting in the central line posterior to the testes, (6) uterus being short and having a few coils.

The genus *Paryphostomum* was proposed by Dietz (1909), with *P. radiatum* as the type species. The species *Distomum radiatum* was created by Dujardin in 1845 on an examination of two examples from the intestine of *Phalacrocorax carbo* in the collection of the Paris Museum. Wedl (1857) described a trematode from the intestine of *P. carbo* under the name of *D. echinatum*, but Dietz

(1910) concluded that *D. echinatum* of Wedl agreed in all respects with *D. radiatum* of Dujardin. Therefore, *D. echinatum* is a synonym of *Paryphostomum radiatum*. Dietz (1909) described another species, *P. segregatum*, from the intestines of *Cathartes urubutinga*, *Sarcorhamphus papa*, *Catharista atrata* and *Oenops aura*. Subsequently to this there is no record of the species of *Paryphostomum*. In Table I are compared the two known species of *Paryphostomum* with the form from *U. hardwickii*.

Table I. *Comparative table of the species of* Paryphostomum.

Host	*P. radiatum* *Phalacrocorax carbo*	*P. segregatum* (1) *Cathartes urubutinga* (2) *Sarcorhamphus papa* (3) *Catharista atrata* (4) *Oenops aura*	*P. indicum* *Uromastix hardwickii*
Size (mm.)	2·3–6·5	5·75 × 0·88	7·5–8·1 × 1·7–2
No. of spines	27	27	42
Marginal spines (mm.)	19 0·74–0·102 × 0·0140–0·0204	19 0·1083–0·1224 × 0·0144–0·0168	32
Terminal spines (mm.)	4 on each side 0·1052–0·1224 × 0·014–0·0204	4 on each side 0·136–0·1428 × 0·0168–0·0216	4 on each side 0·043–0·08 × 0·14–0·[illegible]
Oral sucker diameter (mm.)	0·16	0·17–0·18	0·18
Ventral sucker (mm.)	0·4–0·5 × 0·43–0·66	0·44–0·48 × 0·42–0·45	0·62–0·068
Ratio of oral sucker to ventral sucker	1 : 1·35–4	1 : 3	1 : 3·5
Oesophagus (mm.)	0·23–0·3	0·45–0·65	0·25
Commencement of vitellaria	From the posterior border of ventral sucker	From the posterior border of ventral sucker	From the middle to the posterior border of ventral sucker
Eggs (mm.)	0·08–0·1 × 0·05–0·064	0·086–0·088 × 0·057–0·06	0·04–0·097 × 0·033–0·[illegible]

It is evident from the above table that the present form differs from the other two species of *Paryphostomum* in the following points:

(1) Collar spines being 42 : 32 + 5 + 5 and smaller.

(2) Cirrus sac extending posterior to ventral sucker.

(3) Vitellaria extending anteriorly as far as the middle of ventral sucker.

(4) The larger size of body, and lastly

(5) The host being a reptile.

These points being of sufficient importance to distinguish it from the other two species, it is necessary to create a new species for it, for which I propose the name *P. indicum*. The interest of the species lies in the fact that it is the second species of Echinostomidae obtained from reptiles.

Host: *U. hardwickii*.

Location: Duodenum.

Locality: Northern India.

Stunkardia dilymphosa n.g., n.sp.

The worms are flat, truncated at both ends, and measure 20–20·3 by 5–5·6 mm. In the living condition they exhibited very active movements. They were fixed in their natural size by pressing them a little between two glass slides. The musculature was well developed and the cuticle was thick and smooth.

At the anterior extremity is a wide mouth opening, surrounded by an oral sucker which is somewhat funnel-shaped, measuring 2·52–2·87 by 2·11–2·14 mm. At its posterior border there is a pair of oral pockets, diverging postero-laterally and measuring 0·7–0·9 mm. in length. The ventral sucker situated at the posterior end of the body is very large, measuring 3·88–3·9 by 3·15–3·5 mm. Its structure is very characteristic: its opening is not circular like most of the forms of Paramphistomidae, but its anterior half is semicircular and, posteriorly, it is somewhat like a funnel; the two portions are separated by a septum; moreover its postero-ventral border is produced into two stout muscular processes, the lips, like those present in the genus *Zygocotyle* Stunkard, 1917. The prepharynx and pharynx are both absent. The oral sucker is followed by a somewhat zigzag oesophagus with numerous unicellular glands opening into it. It enlarges posteriorly into an oval oesophageal bulb, measuring 0·75–0·89 mm. by 0·585–0·685 mm. The oesophageal bulb and the oral diverticula are lined with cuticle internally. The musculature of the oesophageal bulb is somewhat different from that of an ordinary pharynx, in that the fibres are not radial but are concentrically arranged. Behind the oesophageal bulb the oesophagus extends for a very short distance, after which it bifurcates into two long intestinal caeca, passing posteriorly into a zigzag manner of a continued **S** and ending somewhat anterior to the ventral sucker, there being four loops on each intestinal caecum. An interesting relation appears to exist between the genital organs and the brackets formed by the loops of the intestinal caeca. Enclosed in the foremost brackets are the vesicula seminalis and the vagina, in the second bracket is the anterior testis, in the third the posterior testis, and in the last one are situated the ovary and the shell gland.

The excretory pore is situated somewhat anterior to the ventral sucker, and leads into an elongated excretory bladder, which increases in diameter as it passes anteriorly and ends a little distance behind the ovary. The anterior end of the excretory bladder gives out two stout branches, which pass postero-laterally towards the end of the intestinal caeca, after which they coil round the latter and pass anteriorly as far as the posterior border of the oral sucker.

Like some of the genera of Paramphistomidae, the lymphatic system is very well developed. It consists of two pairs of longitudinal canals, one of which passes dorsally and the other ventrally. The canals of the dorsal pair start from the centre of the body, immediately anterior to the ventral sucker, and pass anteriorly, approximating each other in front of the excretory

bladder, to diverge again and pass almost parallel anteriorly. The ventral canals arise from the angles between the ventral sucker and the sides of the body and pass anteriorly, approximating each other in front of the excretory bladder, where they diverge towards the sides of the body and pass ventrally to the intestinal caeca, in the anterior region of the body. All the canals can be traced, anteriorly, only as far as the intestinal fork. Throughout their whole length they give out many branches and sub-branches, which ultimately end in small lymph sinuses that are visible all over the body. Special aggregations of these sinuses are seen on either side of the oral sucker, the sides of the body, and the anterior border of the ventral sucker, where especially they present the most picturesque appearance, being arranged like rounded pearls on its periphery.

The testes are situated in the centre of the body, one behind the other, and measure 0·73–0·94 by 0·45–0·7 mm. in size. They are deeply lobed: in one case the anterior one has five lobes and the posterior only four, while in the other the anterior one has four and the posterior five. From the centres of the testes vasa efferentia arise and pass anteriorly, joining together to form a vas deferens which soon enlarges into the vesicula seminalis. The vesicula seminalis is long, measures 1·11–1·16 by 0·12–0·13 mm., and extends ventrally from some distance behind the intestinal fork to the posterior border of the oesophageal bulb, where it opens to the exterior by means of the genital pore. The terminal portion of the vesicula seminalis is differentiated into a muscular structure, the cirrus. There is no indication of a cirrus sac, as was noticed by Stunkard (1917, p. 67) in an allied genus, *Alassostoma*. The prostatic cells opening into the vesicula seminalis lie free in the parenchyma.

The ovary is an irregular body, measuring 0·5–0·53 by 0·39–0·48 mm., and lying some distance anterior to the excretory bladder, to the left of the middle line, close to the ovary. In the central part of the body is a somewhat pear-shaped organ, the shell gland, measuring 0·38–0·5 by 0·15–0·24 mm. A short Laurer's canal can be seen proceeding from the shell gland to the dorsal surface of the body, where it opens to the exterior by a somewhat large opening. Receptaculum seminis is situated near the ovary, and measures 0·5–0·51 by 0·4–0·56 mm. The uterine coils are transverse, and lie in the intra-caecal area anterior to the shell gland. The vagina passes on the left side of the vesicula seminalis and opens into the genital atrium. No eggs are visible in the uterus throughout its course, but in the vagina as many as five to six of them can be seen. The eggs measure approximately 0·132 by 0·117 mm. The vitellaria are very diffuse and consist of very small follicles scattered in the parenchyma, but the vitelline ducts are very prominent and consist of two transverse, stout ducts, meeting behind the shell gland centrally and forming a short duct which passes into the shell gland.

Our knowledge of the amphistomes of Chelonians is very limited. Braun (1901) lists three species: *Amphistoma grande* Diesing, *A. scleroporum* Creplin, and *A. sp.* Bellingham. The last one, although listed by Bellingham (1844)

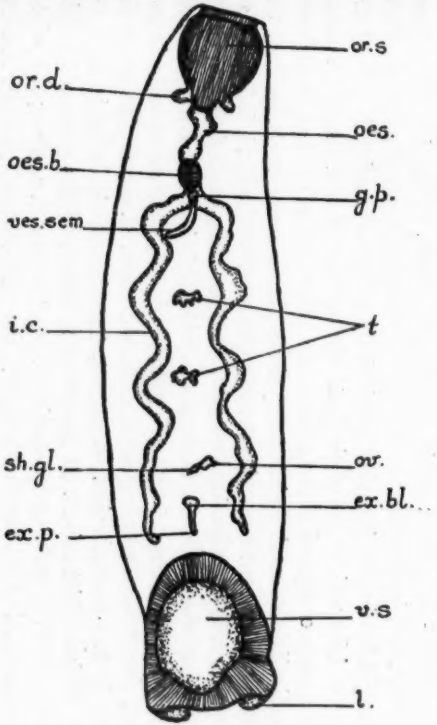

Fig. 3. *Stunkardia dilymphosa* n.g., n.sp.
Ventral view. ×6.

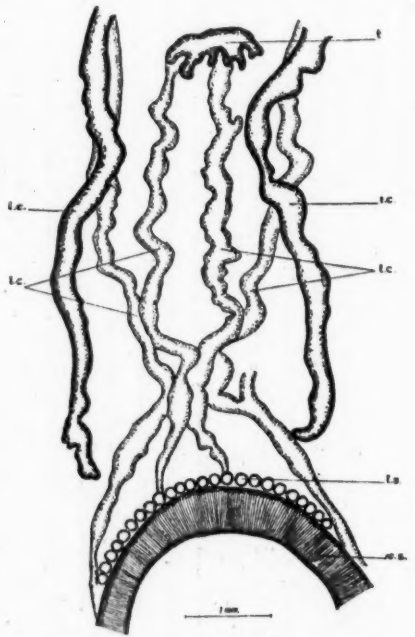

Fig. 4. *Stunkardia dilymphosa* n.g., n.sp.
Lymphatic system.

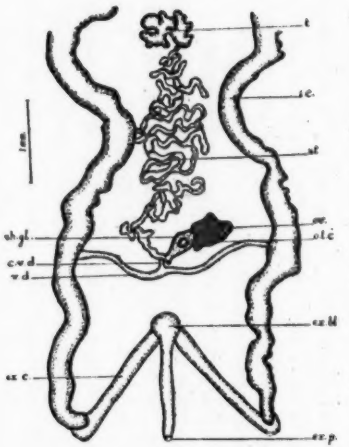

Fig. 5. *Stunkardia dilymphosa* n.g., n.sp.
Female genitalia.

from the intestine of *Chelonia imbricata*, was not described. Braun (1901) supplemented the description of Creplin (1844) by a brief report of a single specimen of *A. scleroporum* from the museum at Greifswald, but the worm was sexually immature, and consequently the observations were limited. *A. grande* was collected by Natterer from the intestines of five species of turtles in Brazil, but the description of Diesing (1839), being confined only to the external features, is of very little taxonomic importance. Looss (1902) described *A. spinulosum* from the intestine of *Chelone mydas*. *A. scleroporum* was described in greater detail by Looss (1902): "The two species *Paramphistomum papilosum* MacCallum, 1916, from the colon of *Chelone mydas* and *P. aspidonectes* MacCallum, 1917, from the oviducts of *Trionyx spinifer* do not belong to the genus *Paramphistomum*, but the descriptions are so imperfect that it is not feasible to attempt a generic disposal of them." Stunkard (1917) described *Alassostoma magnum* from *Pseudemys troosti* and *P. elegans*, and three specimens from an unknown turtle from Marshall. The three specimens obtained from *Chelydra serpentina* were described by him as *Alassostoma parvum* in the same paper, but these were immature. MacCallum (1918) recorded as *Paramphistomum chelidrae* three specimens obtained from *Chelydra serpentina*. Stunkard (1924) obtained mature specimens of *Alassostoma parvum* from the urinary bladder of *Chrysemys picta*, *Pseudemys floridana* and *Chelydra serpentina*. Formerly (1917) he had described both *Alassostoma magnum* and *A. parvum* under a common genus, but as further observations revealed that there were some constant differences between *A. magnum* and *A. parvum* he created a new sub-genus, *Alassostomides*, to include the species *A. parvum*. Looss created (1912) the genus *Schizamphistomum* for the two species *S. scleroporum* and *S. spinulosum*, although he expressed doubt (1912, p. 351) whether two divergent species like these should be included in the same genus. Stunkard (1925) created a new sub-genus, *Schizamphistomoides*, to accommodate it. Evidently, then, all the known species of amphistomes of Chelonians fall into only two genera: *Schizamphistomum* Looss, 1912, and *Alassostoma* Stunkard, 1916, included in the sub-family Schizamphistominae Looss, 1912, Schizamphistominae being characterised by Looss as having two long excretory vesicles which extend singly to the anterior end of the body, and a lymph system composed of three canals on either side of the body which run longitudinally and break up into many sinuses in the region of the suckers. On comparing the present form with the two genera of the family, it is found that it differs from them in having two posterior lips to the ventral sucker, cirrus sac not being present, the ductus hermaphroditicus being absent, the intestinal caeca having four loops, testes being deeply lobed, the oral diverticula not being so close to the oesophagus but somewhat removed, and the lymph canals being only two on each side. It cannot, therefore, be included in the subfamily Schizamphistominae. It comes much nearer to the genus *Zygocotyle* Stunkard, 1917, containing the species *Z. lunatum* and *Z. ceratosa*; the points of agreement being the presence of lips to the ventral sucker, and its division

into two parts, anterior and posterior, the absence of cirrus sac, and the separate openings for the male and female ducts. But it can be separated from it also on account of the oral sucker being terminal instead of sub-terminal, the oral diverticula not being very close to the oesophagus, the oesophageal bulb having concentric rings, and the intestinal caeca being sinuous. Moreover, the excretory and the lymphatic systems have not been worked out in the case of *Zygocotyle*. It cannot, therefore, be included in this genus also. It is, therefore, necessary to create a new genus for its reception, for which I propose the name *Stunkardia* and *S. dilymphosa* for the species.

Generic diagnosis. Zygocotylinae: Middle-sized forms, with terminal oral sucker having two divergent diverticula. Oesophagus with oesophageal bulb having concentric lamellae. Intestinal caeca sinuous. Excretory bladder short, ending anterior to ventral sucker, with arms diverging posteriorly and coiling round intestinal caeca. Lymphatic system with two dorsal and two ventral lymph canals. Testes deeply lobed, intra-caecal, one behind the other. Ovary posterior to testes. Receptaculum seminis and Laurer's canal present. Vitellaria with small and diffuse follicles, vitelline reservoir absent. Uterine coils anterior to ovary, containing a few eggs.

Type species: Stunkardia dilymphosa n.g., n.sp.

Host: *Batagur baska.*

Location: Rectum.

Locality: Rangoon.

Stunkard (1917, p. 79) had suggested that his genus *Zygocotyle* should be placed under a new sub-family, but he did not himself suggest the name. Ward (1917) created the sub-family Zygocotylinae for its reception. Fukui (1929, p. 328) gave a definition of the sub-family Zygocotylinae. The genus *Stunkardia*, having its acetabulum divided into anterior and posterior portions and the latter with two posterior lips, is contained in the sub-family Zygocotylinae, but it differs in some important respects from the genus *Zygocotyle*, which has already been pointed out. In the light of the characters of *Stunkardia* it is necessary to modify the definition of the family Zygocotylinae. Excretory and lymphatic systems are not known in *Zygocotyle*, but it is probable that they resemble those of *Stunkardia mihi*.

Zygocotylinae. Paramphistomidae: Body flat, lanceolate. Acetabulum divided into two parts, the posterior with a pair of appendages; oral sucker with a pair of diverticula, terminal or sub-terminal. Oesophagus with a bulb, with or without concentric muscle fibres. Intestinal caeca straight or sinuous. Testes lobed, one behind the other. Cirrus sac absent. Vesicula seminalis free in parenchyma. Male and female genital pores separate. Uterine coils anterior to ovary, intra-caecal. Vitellaria with small and diffuse follicles, lateral. Excretory bladder small, with arms passing posteriorly up to the ends of the intestinal caeca and then coiling around them. Lymphatic system with a pair of dorsal and a pair of ventral lymph canals. Parasites of birds and reptiles.

Type genus: Zygocotyle Stunkard 1917.

KEY TO LETTERING OF FIGURES.

c. collar; *c.s.* cirrus sac; *c.v.d.* common vitelline duct; *ex.bl.* excretory bladder; *ex.c.* excretory canal; *ex.p.* excretory pore; *g.p.* genital pore; *i.c.* intestinal caecum; *l.* lip; *l.c.* lymph canal; *l.s.* lymph sinus; *m.* mouth; *m.s.* marginal spines; *oes.* oesophagus; *oes.b.* oesophageal bulb; *o.l.c.* opening of Laurer's canal; *or.d.* oral diverticulum; *or.s.* oral sucker; *ov.* ovary; *ph.* pharynx; *p.ph.* prepharynx; *r.s.* receptaculum seminis; *sh.gl.* shell gland; *t.* testis; *t.s.* terminal spines; *ut.* uterus; *v.s.* ventral sucker; *ves.sem.* vesicula seminalis; *vit.* vitellaria; *v.d.* vitelline duct; *vit.s.* vitelline sac.

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(*MS. received for publication* 25. VIII. 1930.—Ed.)

PENNELLA GERMONIA N.SP. A PARASITIC COPEPOD OF *GERMO ALATUNGA*.

By W. HAROLD LEIGH-SHARPE, M.Sc. (Lond.).

(With 1 Text-figure.)

Habitat and Record. By the courtesy, and at the request, of Louis Fage I have examined a specimen of *Pennella* ♀ from the Muséum National d'Histoire Naturelle, Paris, taken at Concarneau, Brittany, on 22. vii. 30, embedded in the skin of an albacore, *Germo*[1] *alatunga* (Gmelin).

The discovery of this animal raises again two questions to which I referred in 1928 (p. 82), whether the *Pennella* from the swordfish, sunfish and tunny are of one species; and whether the horns of all the British Museum specimens are complete (p. 87).

Dimensions. Length: of head and neck 105 mm., trunk 45 mm., abdomen 22 mm., egg-strings (obviously damaged) 35 + mm.; expanse of lateral horns which are curled anteriorly 55 mm., more if they were straightened out (Fig. 1).

Diagnosis. (Following the scheme of the abridged comparison in the table on p. 88, *loc. cit.*) Head almost twice as wide as long, apex slightly concave; papillae large, not markedly in two groups but continuous in the concavity, bright red in colour, a few of them light green. Horns 2*, lateral, standing out at right angles to the head, of enormous length, somewhat bent and curved forwards anteriorly. Ratio of head and neck to trunk 2·5 :1*, ratio of abdomen to trunk 1 : 2. Abdominal appendages much branched, about five filaments (Fig. 1, *A.A.*). Antennules (*A* 1) and antennae (*A* 2) bright scarlet. Anterior end of the neck enlarged.

Determination of species. This specimen does not, as might have been expected, resemble *Pennella filosa* (Linnaeus) in the great majority of the foregoing particulars, as exemplified by the specimens in the British Museum (1928) or as described and figured by other authors, *e.g.* Brian. Unfortunately no host is mentioned for either of the British Museum specimens: on the other hand, it is arguable that an albacore is a very different fish from a tunny; and the discovery of a parasite, generically the same, but specifically widely different, will strongly support the view of those piscatologists who hold that the

[1] I have accepted Jordan's generic name as *Thynnus* is preoccupied for a member of the Lepidoptera.

former exhibits characters sufficiently divergent to warrant its removal from the genus *Thunnus*.

Except for the two characters marked * in the foregoing diagnosis, the present specimen, somewhat unexpectedly, resembles *P. balaenopterae* Koren and Danielssen, a parasite of which there are large numbers at the British Museum (1928), all but one (and that not resembling the present specimen) exactly alike. In view of the fact that this species has succeeded in accustoming itself to a warm-blooded mammal, I do not think it is likely that it would establish itself upon a cold-blooded fish.

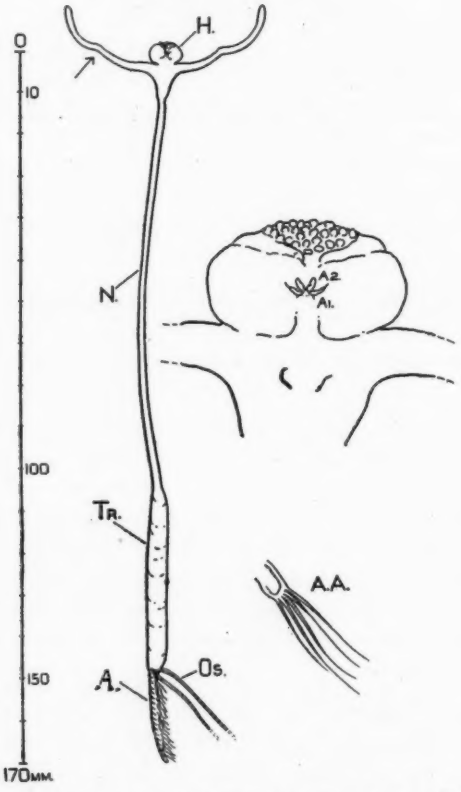


Fig. 1. *Pennella germonia* ♀ n.sp. *H.* = head; *N.* = neck; *Tr.* = trunk; *Os.* = egg-strings; *A.A.* = abdominal appendages; *A.* = abdomen; *A* 1 = antennules; *A* 2 = antennae.

Provisionally, therefore, until the mystery of the discrepancies in the accounts of *P. filosa* alleged to occur on three very different hosts has been cleared up, I prefer to regard this specimen as a distinct species under the name of *P. germonia*.

Note on the horns. At the point marked in Fig. 1 with an arrow one of the horns is broken off but the fragment is included in the phial. I suggest that the horns of all *Pennella* spp. tend to fracture in this way at this specified point, and that their apparently relatively enormous size results from the fact that the horns of the Museum specimens (of *P. balaenopterae* in particular) cease at this point, the condition being due not to fracture, leaving an open hole, as in this specimen, but to attrition.

REFERENCE.

LEIGH-SHARPE (1928). The genus *Pennella* (Copepoda) as represented by the specimens in the British Museum. *Parasitology*, **20**, 79.

(*MS. received for publication* 17. XI. 1930.—Ed.)

THE LOUSE OF ELEPHANTS.

HAEMATOMYZUS ELEPHANTIS PIAGET (MALLOPHAGA: HAEMATOMYZIDAE).

By G. F. FERRIS.

Stanford University, California.

(*From the Molteno Institute for Research in Parasitology, University of Cambridge.*)

(With Plates IV and V and 5 Text-figures.)

It is now slightly more than 60 years since Piaget described as *Haematomyzus elephantis* an extraordinary insect found by him living as an ectoparasite upon an African elephant in the Zoological Garden of Rotterdam. The insect was considered by its describer to be a sucking louse, and this assignment has been accepted by almost all of the few entomologists who have since been privileged to examine specimens. Walker, who in ignorance of Piaget's work, redescribed the species as *Idolocoris elephantis*, regarded it as constituting a new family of Hemiptera Heteroptera near the bed bugs. Since that time no one has questioned the belief that it is a sucking louse and for it Enderlein named a family, the Haematomyzidae, in the Anoplura.

The Molteno Institute for Research in Parasitology has acquired from various sources a considerable number of specimens of this insect. Through the kindness of Prof. G. H. F. Nuttall, the present writer has been permitted to study this material, which has been supplemented by other specimens received as loans through the kindness of the authorities of the British Museum and the Hamburg Museum. Although the condition of the specimens is not favourable for any study of internal anatomy, and the time available to the writer has not in any case been sufficient for such a study, it is possible to present a fairly full analysis of the external morphology of the species, together with some fragmentary information as to immature stages.

It is clear, from this study, that *H. elephantis* is not a sucking louse.

Systematic history of the genus and species.

There being but one species in the genus, the generic and specific references for the most part coincide. They are therefore considered together.

1869. *Haematomyzus elephantis* Piaget, *Tijdschr. voor Entomol.* **12**, 254.

The original description of the genus and species.

1871. *Idolocoris elephantis* Walker, Richter, *Hardwicke's Science Gossip*, pp. 131–132, fig. 67.

Richter quotes the description, prepared for him by Walker and therefore to be credited solely to the latter, of *Idolocoris elephantis*, new genus and species. The describer was ignorant of Piaget's earlier description. The statement is made that the genus forms the type of a new family of Hemiptera Heteroptera, although the family is not formally named. The description of the species is really good.

1871. *The Elephant Parasite*, T. G. Denny, *ibid.* p. 185.

Records merely that his father, author of the *Monographia Anoplurorum Brittaniae*, possessed specimens of this species.

1871. *Idolocoris elephantis* Walker, Richter, *ibid.* p. 211.

Irrelevant discussion of the credit for the discovery of the new genus and species.

1871. *Phantasmocoris*, F. Buchanan White, *ibid.* p. 234.

Notes that *Idolocoris* Walker is a homonym of *Idolocoris* Douglas and Scott and proposes a new generic name.

1871. *Haematomyzus elephantis* Piaget, Günther, *ibid.* p. 278.

Re-establishes the name *Haematomyzus elephantis* Piaget.

1872. *Haematomyzus elephantis* Piaget, Newman, *Entomologist*, **6**, pp. 465–470, fig.

Reproduces the figure of Richter, accompanied by much irrelevant discussion.

1880. *Haematomyzus proboscideus* Piaget, *Les Pédiculines*, pp. 658–660, pl. LIV, figs. 2–2 *h*.

For no apparent reason renames the species.

1897. *Haematomyzus proboscideus* Piaget, Mégnin, *Bull. Mus. d'Hist. Nat.* Paris, No. 5, pp. 167–169, figs.

Adds nothing to the knowledge of the insect.

1904. *Haematomyzus proboscideus* Piaget, Enderlein, *Zool. Anzeiger*, **28**, pp. 136–137.

Names the family Haematomyzidae of the order Anoplura for this species.

1908. *Haematomyzus elephantis* Piaget, Dalla Torre, Anoplura in Wytsman's *Genera Insectorum*, p. 19, fig. 13.

Listed in catalogue, accompanied by crude figure.

1908. *Haematomyzus paradoxus* Lahille.

Unable to trace the reference.

1910. *Haematomyzus proboscideus* Piaget, Mjöberg, *Ark. för Zool.* **6**, pp. 181–183, fig. 93.

Records the species and gives a largely inaccurate figure of the tracheal system.

1910. *Haematomyzus elephantis* var. *sumatranus* Fahrenholz, *Zool. Anzeiger*, **35**, p. 714.

Preliminary diagnosis of a supposed new sub-species.

1910. *Haematomyzus elephantis* var. *sumatranus* Fahrenholz, *Jahresb. des Niedersächs. zool. Ver. zu Hannover* 58–59, Abt. D, p. 67.

Repetition of the preceding description.

1916. *Haematomyzus elephantis* Piaget, Ferris, *Proc. California Acad. Sci.* (Series 4), **6**, p. 185.

Lists in catalogue.

1916. *Haematomyzus elephantis* Piaget, Fahrenholz, *Jahrb. d. Hamburg. Wiss. Anstalten*, **34**, Beiheft 2, p. 6.

Records the two supposed sub-species from material in the Hamburg Museum.

1919. *Haematomyzidae*, Nuttall, *Parasitology*, **11**, p. 333.

Notes that the mouth-parts are aberrant and require to be studied to render the systematic position of the family secure.

Previous records.

Recorded by Piaget from African elephant in the Zoological Garden of Rotterdam. By Richter from a single specimen from elephant in Ceylon. By Denny from specimens from unspecified kind of elephant in a travelling menagerie. By Megnin from unspecified elephant species from the Zoological Garden of Paris. By Mjöberg from Indian elephant, without indication of locality, from specimens in the Hamburg Museum. By Fahrenholz from specimens in the Hamburg Museum from Indian elephant in the Zoological Garden of Hamburg and from Sumatran elephant.

Specimens examined.

A. Six slides from the Piaget collection, received on loan through the kindness of Major E. E. Austen of the British Museum which now owns this collection. These slides were remounted by me in order to render them suitable for study. The data concerning them are as follows:

One slide, containing a male and a female, labelled "*Haematomyzus proboscideus* P. sur un jeune éléphant d'Afrique." These may be regarded as the types of the species.

One slide, labelled by the same specific name and with merely the data, "sur une éléphant jeune." Probably co-type material.

One slide with the same specific name and with merely the data, "sur un Elephas."

Three slides, labelled by the same specific name, each containing two females and one male and each with the data "sur un éléphant de Sumatra." Apparently these were not in the hands of Piaget at the times when he recorded the species and are therefore not to be considered as type material.

B. Ten slides and four alcoholic specimens from the collection of the Hamburg Museum received on loan through the kindness of Dr E. Titschack, Director of that Museum. The data concerning these specimens being as follows:

This material includes on slides five females, four males and a mount of eggs, all bearing a label "Coll. A. Poppe (3. vii. 1901)" and variously "von Elephas sumatranus 1901" and "von Elephas indicus." It apparently does not include the types of Fahrenholz' *H. elephantis* var. *sumatranus*. The alcoholic material mounted by me includes one male, two females and an immature specimen. These are the specimens recorded by Fahrenholz (1916) as "von sumatranischen Elefanten, S. A. Poppe ded. 25. xii. 1901."

C. Numerous specimens, upon which the accompanying morphological studies have been based, in the Nuttall Collection of the Molteno Institute of Parasitology. These include three lots from Indian elephant, Rangoon, Burma "12. v. 1913. Dr H. H. Marshall," "12. vii. 1919. Dr H. H. Marshall," and "21. vii. 1919. Mr Rogers." One lot is indicated as from "*Rhinoceros indicus* (*unicornis*), Schönbrunn, Vienna Museum coll. 1885."

Hosts.

Apparently all the specimens thus far taken of this species have been from animals in captivity. It is evidently normal to the Indian elephant, and whether the original record from African elephant, and above all, that from rhinoceros, indicate anything more than purely chance occurrences in zoological gardens remains to be determined.

Supposed sub-species.

I am unable to see any justification whatsoever for the supposed subspecies *sumatranus* named by Fahrenholz. It was based entirely upon slight differences in measurements.

Morphology.

Size.

Total length of female, in expanded condition mounted on a slide, 3 mm. of which the head constitutes 1 mm. Male 2 mm., the head 0·75 mm. The specimens of the supposed variety *sumatranus*, which were indicated by

Fahrenholz to be smaller, are of approximately the same lengths when prepared in the same manner.

The head.

General form and structure. The form of the head recalls that of the rhyncophorous Coleoptera, the anterior portion being produced into a slender, down-curved beak. The fusion of the head sclerites has been carried so far that none of the individual plates can be detected, and it is only from the position of the eyes, the antennae and the mouth-parts that any deductions as to the homologies of parts can be made. The whole structure is heavily sclerotic, except for the ventral side of the head, which is slightly membranous. There are no epicranial or other sutures visible.

It is evident that the proboscis is formed by the elongation of all the parts of the head capsule lying anterior to the base of the antennae, the trophi and the oral opening being at the apex of this structure. The antennae arise from the sides of the head at the base of the proboscis.

The one-faceted eyes are in the position at the sides of the head which is normal to the compound eyes, of which they may be assumed to be vestiges, and there are no ocelli.

From the dorsal, posterior border of the head there arises a very large and conspicuous median apophysis, which projects into the thorax and is of a sharply conical form. It appears to be hollow and to open at the surface by a narrow slit. The occipital foramen is crowded to the apparent ventral side of the head and its anterior margin is marked by a pair of strongly sclerotic median lobes, between which a pair of apophyses, later to be described, are firmly attached.

The antennae.

The antennae (Fig. 3 C) are five-segmented and entirely simple in their structure. Their most striking feature is the length of the first segment, which is nearly as long as the next three segments together. The conspicuous thickenings of the walls, which are present in the antennae of all ectoparasitic forms, are strongly developed. The circular pits or sensoria, which are present on the two terminal segments of most, if not all, of the sucking lice, are here lacking.

The mouth-parts.

At the extreme apex of the proboscis there appear a number of heavily sclerotic and deeply pigmented teeth, some of which are dorsal, some ventral and some lateral. The entire structure which bears them is extremely small and the deep pigmentation renders it difficult, or even impossible, to see the more delicate, membranous connections. However, it is possible to dissect away the parts sufficiently well to reveal the fundamental features clearly.

The proboscis terminates dorsally in a flat plate (Fig. 2 B), which is rounded and slightly bilobed at the apex, with each of these lobes bearing a delicate, setiform structure. From the dorsal aspect of the plate there arise three pairs of flat, leaf-like teeth of which the first pair are close to the anterior margin, are small, irregularly lobed and lie in pits in the derm. The members of the

second pair are larger and are likewise contained within a depression. The members of the third pair are still larger and may even project somewhat beyond the sides of the proboscis.

The ventral termination (Fig. 2 A) consists of a pair of heavily sclerotic, elongate plates which are slightly separated medially by an ill-defined smaller plate. The lateral plates each bear posteriorly two flat, leaf-like teeth and culminate anteriorly in a rounded lobe (Fig. 2 D) that bears evidence of being composed of two segments each of which bears a single seta. This whole structure appears to be to some extent movable.

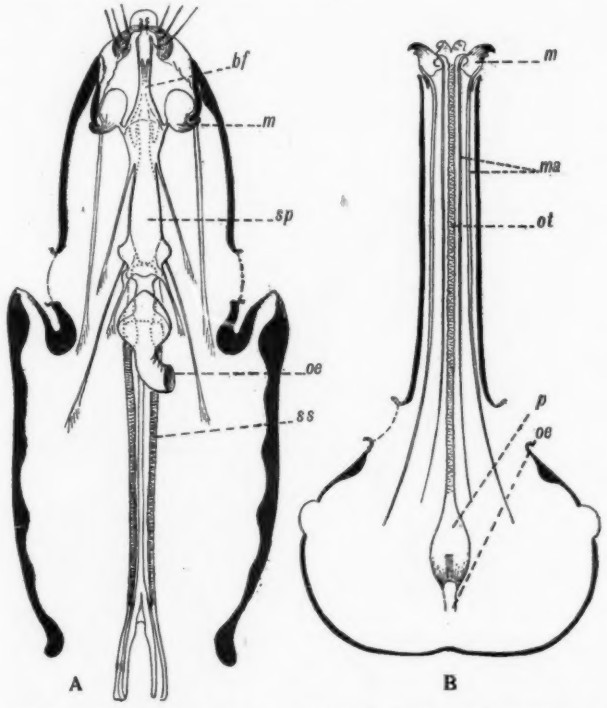


Fig. 1. A. Mouth-parts of *Haematopinus suis* (Linnaeus). B. Mouth-parts of *Haematomyzus elephantis* Piaget. *bf*, buccal funnel; *m*, mandible; *ma*, mandibular apodeme; *oe*, oesophagus; *ot*, oral tube; *p*, pharynx; *sp*, sucking pharynx; *ss*, stabber sac.

The sides of the proboscis, apically, are formed by a mass of small, curved teeth. Manipulation with a sharp needle demonstrates that those of each side are parts of a single, movable structure. This can be dissected off and viewed from the lateral aspect has the appearance shown in Fig. 2 C.

It is with these lateral structures that we are principally concerned. If they are carefully removed from the head, it can be seen that the structure of which they are a part bears a pair of apodemes that can be traced the full length of

the proboscis and into the cavity of the head. Furthermore, it can be demonstrated that the entire structure pivots upon a condyle which arises as a knob-like structure from the inner wall of the dorsal apical plate.

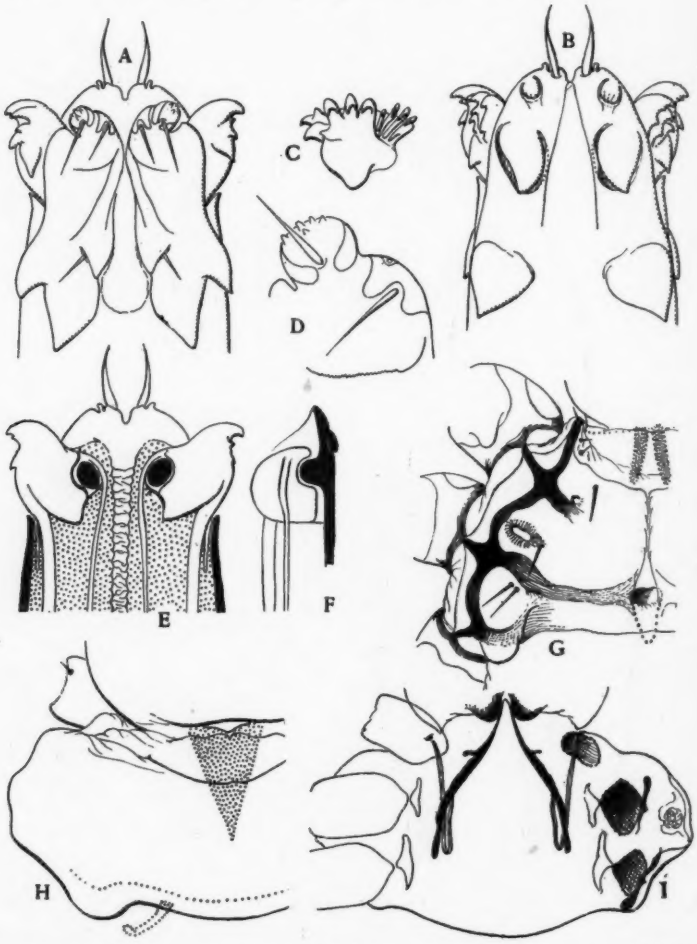


Fig. 2. Structural details of *Haematomyzus elephantis*. A. Ventral aspect of apex of proboscis. B. Dorsal aspect of same. C. Lateral aspect of mandible. D. Detail of palpus. E. Apex of proboscis with dorsal plate removed to show mandibles with their condyles and apodemes. F. Longitudinal section, diagrammatic, of apex of head to show position of mandibular condyle. H. Dorsum of thorax. I. Venter and internal apophyses of thorax, *Haematopinus suis*. G. Dorsum of thorax.

Arising between the bases of these two teeth-bearing structures there appears a rather conspicuous, somewhat sclerotic and wrinkled tube, which is obviously the oral tube and which extends posteriorly into the head capsule,

where it expands into a simple and somewhat sclerotic bulb, from which it proceeds as the delicate oesophagus. The bulb is evidently the pharynx. No salivary duct can be detected.

Lying within the proboscis there can be seen four more very delicate apodemes, the apical termination of which is obscured by the heavy pigmentation. They almost certainly insert upon some portion of the movable ventral plates.

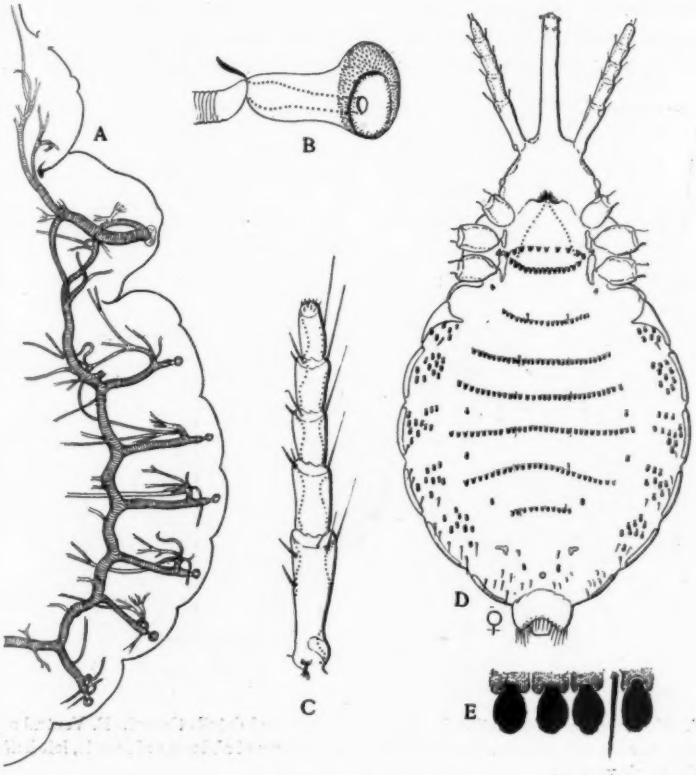


Fig. 3. *Haematomyzus elephantis.* A. Main trunks of right half of tracheal system. B. Spiracle. C. Antenna. D. Ventral aspect of female. E. Modified setae of the venter.

So much then for the bare description of what is present. It will be noted that these structures are fundamentally simple and that there is no series of complicated organs such as are present in the head of a typical sucking louse (Fig. 1 A). *Most particularly is it to be noted that the stabber sac with its contained piercing stylets, which is the most distinctive feature of the mouth-parts of a sucking louse, is here entirely lacking.* There are, in fact, no piercing organs whatsoever.

There remains the task of interpreting what is to be seen. As to this interpretation, it appears to the writer that there is but little room for difference of opinion.

That the toothed, lateral structures are mandibles is so obvious that there would seem to be no need to belabour the point. Their position, their form and even the transfer of the cutting or tearing edges to the outer side are directly parallel with the conditions found in the rhyncophorous beetles. A curious parallelism, indeed, but nothing more and to be stressed merely as facilitating an understanding of the conditions. The most curious feature of these mandibles is that of the position of the condyle (Figs. 2 E, F) which has moved inward

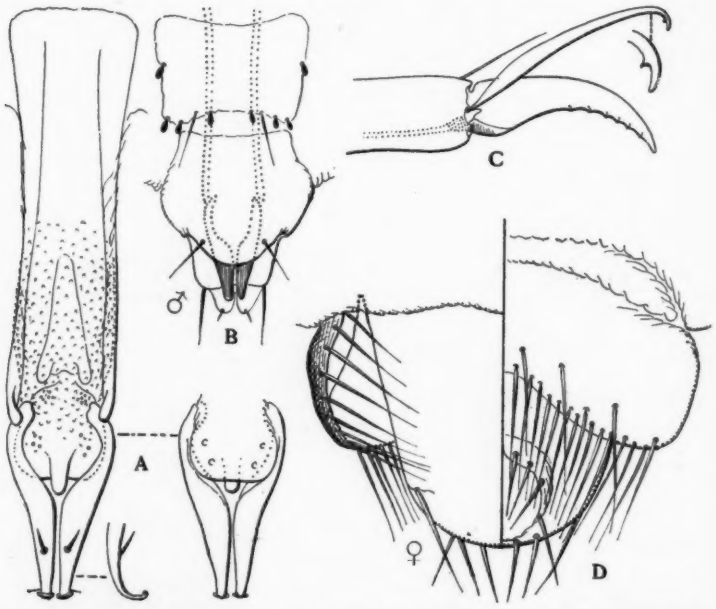


Fig. 4. *Haematomyzus elephantis.* A. Genitalia of male and details thereof. B. Ventral aspect of apex of abdomen of male. C. Apex of tarsus. D. Apex of abdomen of female, left half dorsal, right half ventral.

until it rises from the apparent dorsal wall of the head instead of from the lateral wall. Even this is paralleled to some extent in the shifting of the condyles in the weevil genus *Balaninus*.

So much is clear. That the dorsal plate can rightly be interpreted as the labrum is doubtful. Against such an interpretation is the fact that the mandibular condyles arise from it. In all probability the labrum and clypeus are lacking or are reduced to a narrow median area, and this plate is merely the apical termination of the frons, with the inclusion, perhaps, of a portion of the genae.

Also in connection with the ventral plates there is doubt. The apical, segmented lobes seem obviously to be the vestiges of palpi, but whether the bases from which they arise are the maxillae, with the labium represented by the ill-defined median plate, or whether the whole structure is the labium with the maxillae lacking, as they are supposed to be in some of the Mallophaga, is open to question. Evidence to settle the point might be obtained from the points of insertion of the four delicate apodemes which have been mentioned, but it has not been possible to trace these to their terminations. A study of the parallel developments in rhyncophorous beetles, if one dared to press conclusions far enough, might settle the point.

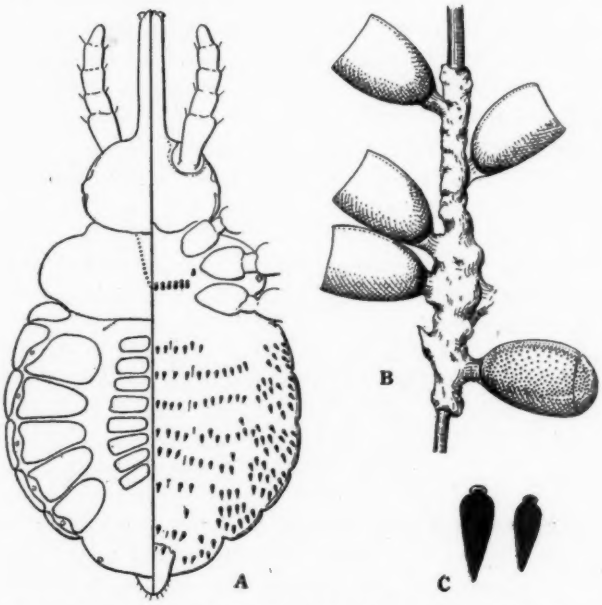


Fig. 5. *Haematomyzus elephantis.* A. Nymph, legs omitted. B. Eggs. C. Modified setae of the venter.

It is not the desire of the writer to be misunderstood as intimating that this insect has anything to do with the rhyncophorous beetles. It is a form with incomplete metamorphosis. But the curious parallelism between its head form and oral structures and those of such beetles contained the hint which led him to break away from all of the prepossessions which had caused him, like earlier workers, to accept the insect as a sucking louse and to enquire further into its structure. It would seem that in all probability this parallelism extends to the method of functioning of these parts as well as to their structure.

Somewhere in the vast literature of the rhyncophorous beetles there must be information concerning their exact mechanism of feeding, but it is not to be

found in general discussions of the group. Bearing in mind the extraord nary slenderness of the beak in some of these forms and the fact that down that beak must pass the apodemes of the mouth-parts, as well as the oral tube, we may conclude independently that many of these insects must ingest their food as a liquid by a sucking action, the mandibles being used to tear the food material and free the juices.

Such, in all probability, is the method of functioning in *H. elephantis*. Other writers, taking for granted the presence of the piercing stylets, have assumed that the elongation of the head is for the purpose of permitting the lengthening of these stylets in order that they may pierce through the thick skin of the host to reach the blood supply. Such an hypothesis can no longer be maintained. It would seem that the toothed mandibles are used to lacerate the derm, much as the terminal hooks are used in the ticks, that the blood is started flowing by such boring and the oral opening is merely applied to the wound and the blood sucked up by a pumping action of the pharynx. One may suspect that the insect probes with its beak into the folds of the skin of its host and that the apical teeth assist it to cling on a relatively hairless body.

The thorax.

The thorax is short and broad and dorsally is composed for the most part of a single, heavily sclerotic plate which bears no segmental lines or indications of divisions. A narrow anterior rim, which is pale and but slightly sclerotic, may be assumed to represent the pronotum, while the single plate may be assumed to represent the fused meso- and metanota. The lateral and posterior margins of this plate are sharply angulate and present a strongly sclerotic ventral marginal fold. There are no dorsal structures of any sort except a pair of short apophyses projecting into the body from the posterior border of this plate.

The legs attach on the ventral side, well back from the margin, the pleural condyles, except in the case of the first pair, arising from small, detached sclerotic areas (Fig. 2 I). It may be assumed that the region from these condyles to the lateral rim represents the true pleural region of the thorax and that the dorsal shield is the true notum.

There is but a single pair of thoracic spiracles, these being on the ventral side of the overhanging marginal shelf and almost directly in line with the mesothoracic coxal condyles.

There is no sternal plate, but at the mesal point of each middle and posterior coxa is a small, detached sclerotic area.

The ventral apophyses, which as the furcae are common to most pterygote insects and which in the Anoplura arise from the sternum near the anterior coxae, are here represented by an internal framework of peculiar position. It is, in fact, possibly not directly or at least entirely homologous with these apophyses at all. Arising from the median ventral lobes of the occipital foramen of the head, where they are solidly attached, are a pair of slender, strongly

sclerotic apophyses which pass posteriorly into the thorax as far as the level of the middle coxae and there divide. One short arm ends blindly. The other rises dorsally in a curve and then passes forward again to meet the coxal condyle of the corresponding anterior leg and by its ending in the body wall to form the pleural condyle upon which this coxa moves. A most curious arrangement, but so clearly demonstrated in several dissections that there is no doubt as to its occurrence.

It is not the intention here to discuss at length the thorax of the sucking lice, such a discussion being intended for another paper. But for purposes of comparison, a figure representing a portion of the dorsal aspect of the thorax of *Haematopinus suis* (Linnaeus) is presented. It is the writer's contention—which he will elsewhere elucidate and defend—that in the sucking lice the true notum is vestigial or even entirely suppressed, being at the most represented only by the narrow median line and the notal apophysis (Fig. 2 G) when this is present. All the remainder of the apparent notum appears to be composed of the fused pleural plates, the strengthening ribs being of the nature of phragmata formed along the lines of fusion of the epimera and episterna.

If the interpretations here given for the parts of the thorax be correct it is evident that there is a wide difference between *Haematomyzus* and the sucking lice.

The legs.

The legs of *Haematomyzus elephantis* depart widely from the form which is common to all the sucking lice. They are extremely slender, being in fact of a distinctly cursorial type, and entirely lack any provision for clinging to the hairs such as is characteristic of the short, stout legs of the Anoplura.

The attachment of the coxae to the thorax has already been discussed. The trochanters and femora present no features of interest. The tibiae are divided by a pseudo-articulation into two parts. Although these parts are sharply marked it is evident that there is no true articulation and no muscles are provided for the operation of the terminal portion.

The tarsi are more than half as long as their tibiae, are slender and cylindrical and terminate in a single stout claw which bears small teeth on its inner face. In addition to this claw (Fig. 4 C) there is another apical structure in the form of a curved, flattened seta which arises from beside the claw. The suggestion that this is in fact a second claw is inescapable because of its position, but it possesses no unguitractor plate and is entirely independent of the true claw.

The abdomen.

The abdomen in both sexes is broad and flattened and rather strongly sclerotic. There is sufficient difference between the sexes to make it desirable to deal with them separately.

Female. The segmentation is clearly indicated and there seems no particular reason for doubt as to the numbering of the segments. If we assume

that the genital opening of the female is between the eighth and ninth segments, the count of the pleurites from this point indicates that the true first segment is suppressed, the first apparent segment being morphologically the second. The ninth and tenth segments are closely fused into a small, truncate cone which is partially enveloped by the gonopophyses, can be telescoped within the body and bears the anus on the ventral side at the apex (Fig. 4 D). The spiracles are borne on the third (second apparent) to eighth segments on the dorsal side at the margin of the pleurites.

The pleurites are well-developed as distinct plates of more or less quadrate form on the morphological second to eighth segments, those of the first two segments being larger, the first extending somewhat on to the dorsal side, the second sending out a short arm on the ventral side. Dorsally on the third to seventh segments and separated from the pleurites by a narrow membranous area there is on each segment a large, triangular, sub-marginal plate. While almost the whole dorsum is somewhat sclerotic there are present a series of sharply defined and deeply pigmented median plates. That of the eighth segment is a large plate extending from one pleurite to the other. Those of the other segments are small and narrow. Their segmental distribution is not clear. The plate belonging to the morphological second segment is obviously marked off by an intersegmental furrow, but there remain seven plates to allocate to five segments, and the segmental folds are not sufficiently well defined to indicate to which they belong. At each end of the plate belonging to segment two is a small, detached, weakly sclerotic area bearing a pair of flattened setae.

On the ventral side the derm is squamate-reticulate and is entirely membranous except for two minute areas which are apparently on the posterior border of the seventh segment. There are six well-defined, transverse rows of short, flattened setae (Fig. 3 E) which apparently belong to the second to seventh segments, and at the lateral margin of each segment is an irregular group of these mingled with small, slender setae.

The gonopophyses arise as lobes from the posterior border of the eighth segment and are continuous across the ventral side, their margin being beset with long hairs. The genital opening, as has been demonstrated by probing of unmounted specimens, is concealed by the flap formed by these lobes. Within the body can be detected a small, circular, sclerotic area which probably surrounds the opening of the spermathecal duct into the uterus. The duct itself is too delicate to remain in cleared specimens.

Male. The abdomen of the male, while resembling that of the female in general form, differs markedly in appearance because of the enlargement and fusion of most of the tergal plates into a single large plate. This fusion involves the marginal and median plates of the morphological third to seventh segments, and the lines between the various plates are but faintly, although definitely, indicated. The first two and the last two median plates remain separate.

The ninth and tenth segments have somewhat the same conical form as in the female, the anus opening dorsally at the apex of the cone and being sur-

rounded by a sclerotic setigerous ring. The genital opening is immediately below the anus, in the ninth segment. The ventral side of the eighth and ninth segments bears a small, median genital plate (Fig. 4 B).

The genitalia of the male (Fig. 4 A) are of the type which is common to both the biting and the sucking lice. As in these groups, they consist of an extrusible membranous sac, the preputial sac or vesica penis, which bears numerous small, sclerotic placodes; the long basal plate, which forms a part of the wall of this sac; and the parameres which articulate to the apex of the basal plate and are basally continuous with the walls of the sac. The apex of the sac is slightly sclerotic, and within its walls is to be seen a narrow, elongate, sclerotic piece. The tips of the parameres are sharply curved upward and slightly back from their apices is a single stout, retrorse seta.

The tracheal system.

The position of the spiracles has already been pointed out. They are quite simple (Fig. 3 B), consisting apparently of a small chamber, the walls of which are beset with minute spines, this chamber opening into a thick-walled tube which communicates with a narrow opening that leads into the trachea. The closing apparatus, represented in cleared preparations by a minute, sclerotic bar, seems to be quite the same as has been described by various authors for the sucking lice.

The tracheal system (Fig. 3 A) consists of a pair of large lateral trunks connected by a single large commissure in the eighth abdominal segment and communicating with the spiracles by lateral branches. The main trunks are relatively little branched, the greater part of the branches arising from a common stem on each spiracular branch. The trunk rising from each thoracic spiracle is even larger than the lateral trunk, to which it unites by a triangular connection. It is not possible in the material at hand to identify more than the one transverse commissure. The entire arrangement is very similar to that in the genus *Haematopinus* of the sucking lice.

IMMATURE STAGES.

The material at hand contains a single specimen, belonging to the Hamburg Museum, which represents an immature stage. In its general features (Fig. 5 A) this is very similar to an adult. The mouth-parts and antennae are the same. The legs are somewhat shorter and stouter. The tergal plates are as in the adult female, except that the median plates are divided by a median line into two parts. The genitalia are, of course, not developed and the derm is in general less sclerotic than in the adult. The modified setae of the ventral side are proportionately much larger than in the adult and are cuneiform (Fig. 5 C).

A single slide, in the collection of the Hamburg Museum, contains a portion of a hair bearing several eggs and labelled as from "*Elephas sumatranus.*" These eggs (Fig. 5 B) are of the characteristic type found in both the biting and sucking lice.

Systematic position of *Haematomyzus*.

As will be clear from the foregoing account, especially that of the mouth-parts, *H. elephantis* can no longer be allowed to remain with the sucking lice. But ejected thus rudely from its abiding place of more than sixty years, it must be re-established somewhere. At the present time a certain amount of restraint in dealing with it is necessary.

There is first to be considered the possibility that it is the hitherto entirely unknown "missing link" between the biting and sucking lice. But it is clear that such an assignment is untenable. *The mouth-parts are strictly mandibulate.* Their only real peculiarities consist of the shifting of the mandibular condyles and of the cutting face of the mandibles themselves. And, as has been pointed out, this is paralleled in every way among such forms as the rhyncophorous beetles. The loss of the labrum is nothing unusual. And whatever interpretation may be given to the parts composing the ventral floor of the oral region, it can be paralleled elsewhere among mandibulate forms.

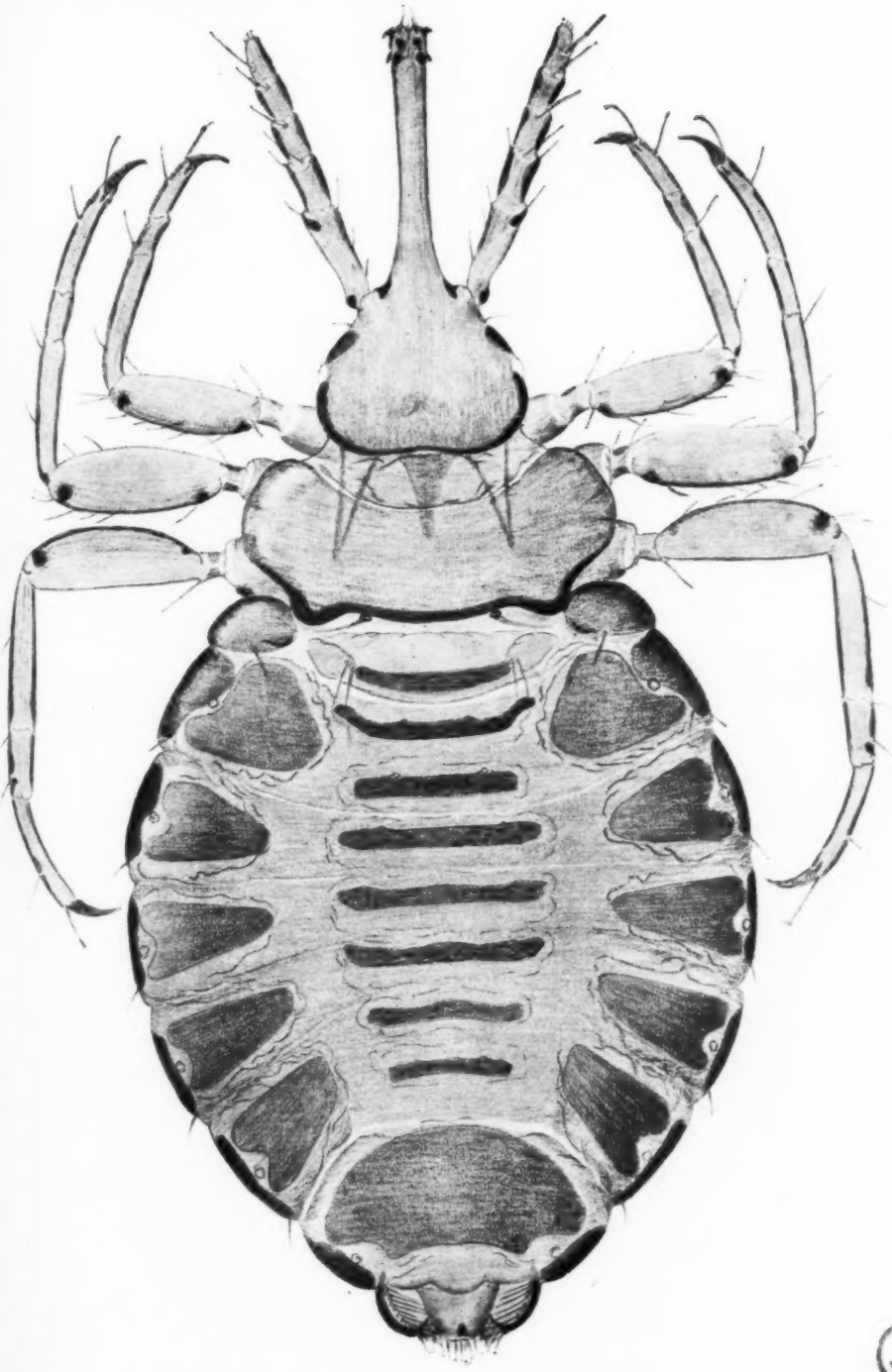
The mouth-parts, in fact, show not the slightest indication of a transition toward those of the sucking lice. Although functionally of a sucking type they contain no piercing mechanism.

In certain respects, however, the insect recalls clearly the general characteristics of the Mallophaga and Anoplura. The five-segmented antennae are characteristic of both groups. The distribution of the spiracles, one thoracic pair and six abdominal pairs on the third to eighth segments, is that which is most common in both these groups. The tracheal system itself might be taken almost directly from a species of *Haematopinus*. The genitalia of the male might come from a member of either order.

On the other hand the thorax is characteristic of neither the Mallophaga nor the Anoplura, and there is certainly nothing in the Mallophaga having even the most remote resemblance to the curiously modified mouth-parts of *Haematomyzus*. Nor are the legs similar to anything which occurs in the Mallophaga, although the differences may possibly be regarded as purely adaptive.

There exist at the present time no morphological studies of any species of Mallophaga that are adequate for our needs in a situation such as this. It may be that such studies will show the impossibility of associating this species with the Mallophaga. However, the type of development and the broad morphological features associate the species in a general way with the biting lice.

It is the writer's belief—at present—that if we accept the Mallophaga and Anoplura as groups which have arisen from a common stem, as they apparently have, we probably see in *H. elephantis* the sole surviving vestige of a third branch from that same stem and equivalent in rank to either of the others. Consequently, if the Mallophaga and Anoplura be regarded as sub-orders of a single order, an arrangement which has some arguments in its favour, *Haematomyzus* would constitute a third sub-order of this group. Or, if it be preferred



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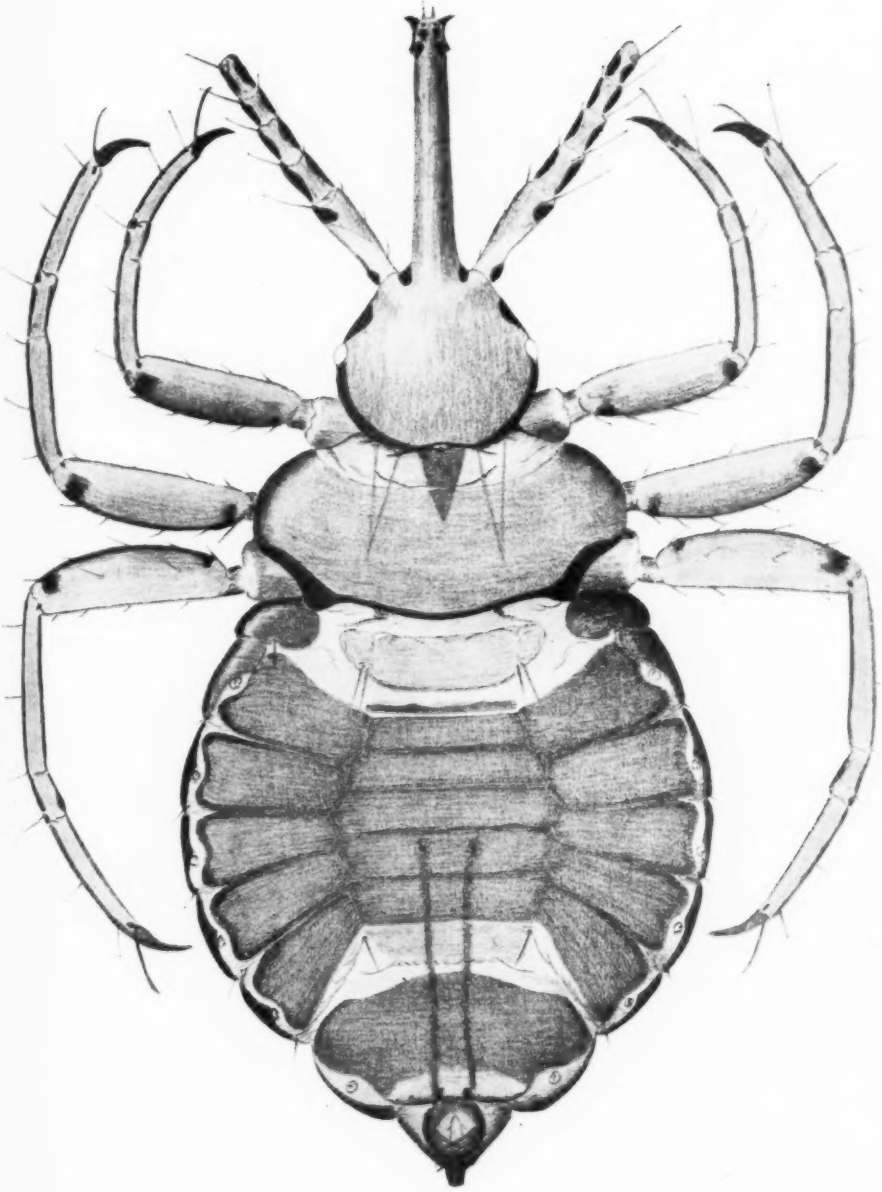

to regard the Mallophaga and Anoplura as distinct orders in accord with the commonly accepted practice, a new order would need to be named for this one species.

But the naming of a new order is not to be undertaken lightly. Even if it eventually be regarded as justifiable it can very well wait until there exist more thorough comparative morphological studies of the Mallophaga and Anoplura than we have at present and until we have more information concerning *H. elephantis* itself.

As matters stand the only justifiable procedure is to attach the genus *Haematomyzus* to the Mallophaga, it being a louse with biting mouth-parts. But within that order its unique position should be recognised by placing it as a sub-order, for which I propose the name Rhyncophthirina. This sub-order will contain the single family Haematomyzidae Enderlein. And thus *Haematomyzus* may settle down for at least a time in a new resting place.

DESCRIPTION OF PLATES IV AND V.

Haematomyzus elephantis Piaget, female.
Haematomyzus elephantis Piaget, male.

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PAGES

DOBELL, CLIFFORD. Researches on the Intestinal Protozoa of Monkeys and Man. IV. An experimental study of the *Histolytica*-like species of *Entamoeba* living naturally in Macaques 1

WIGGLESWORTH, V. B. Digestion in *Chrysops silacea* Aust. (Diptera, Tabanidae) 73

ROY, D. N. and MAYNE, BRUCE. A Note on the Presence of Supernumerary Lobes in Salivary Glands of Anopheline Mosquitoes. (With Plate I) 77

JOBLING, B. A New Species of the Genus *Raymondia* Frauenfeld (Diptera Pupipara, Streblidae) with a note on *Raymondia quadriceps* Jobling and *R. bedfordi* Ferris. (With 2 Text-figures) . . 79

MOGHE, M. A. A Supplementary Description of *Lytocestus indicus* Moghe (Syn. *Caryophyllaeus indicus* Moghe 1925. Cestoda). (With 6 Text-figures) 84

BROWN, F. J. Some Fresh-water Larval Trematodes from Cheshire. (With Plates II and III including 15 figures) 88

BHALERAO, G. D. Two new Trematodes from Reptiles: *Paryphostomum indicum* n.sp. and *Stunkardia dilymphosa* n.g., n.sp. (With 5 Text-figures) 99

LEIGH-SHARPE, W. HAROLD. *Pennella germonia* n.sp. A Parasitic Copepod of *Germo alatunga*. (With 1 Text-figure) . . . 109

FERRIS, G. F. The Louse of Elephants. *Haematomyzus elephantis* Piaget (Mallophaga: Haematomyzidae). (With Plates IV and V and 5 Text-figures) 112

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